RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 21–22, 2016

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

Contents

[Note: The latest Human Gene Transfer Protocol List can be found on the Office of Biotechnology Activities website at http://osp.od.nih.gov/office-biotechnology-activities.]

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NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting[1]

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The Recombinant DNA Advisory Committee (RAC) convened for its 146th meeting at 8:30 a.m. on June 21, 2016, at the National Institutes of Health (NIH), Building 35, Conference Room 620/630, Bethesda, Maryland. Dr. Richard Whitley, Acting RAC Chair, presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 4:45 p.m. on June 21, 2016, and from 8:30 a.m. until 12:40 p.m. on June 22, 2016. The following individuals were present, either in person or by teleconference, for all or part of the June 2016 RAC meeting.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Paula Cannon, University of Southern California *(via teleconference)*
Mildred Cho, Stanford University School of Medicine
William Curry, Harvard Medical School
Kevin Donahue, University of Massachusetts Medical School *(via teleconference)*
Angelica Hardison, Augusta University *(via teleconference)*
Patrick Hearing, Stony Brook University
Howard Kaufman, Rutgers *(via teleconference)*
Douglas McCarty, Ohio State University College of Medicine
Joseph Pilewski, University of Pittsburgh
Lainie Ross, University of Chicago Medical Center
Richard Whitley (Acting RAC Chair), University of Alabama, Birmingham, School of Medicine
Dawn Wooley, Wright State University
Laurie Zoloth, Feinberg School of Medicine, Northwestern University

NIH Office of Science Policy (OSP), Division of Biosafety, Biosecurity and Emerging Biotechnology Policy (BBEBP)

Jessica Tucker, Office of the Director (OD), NIH

Nonvoting Agency Representatives

Kristina Borror, Office for Human Research Protections, U.S. Department of Health and Human Services
Denise Gavin, Office of Cellular, Tissue, and Gene Therapies, U.S. Food and Drug Administration (FDA)
Carrie Wolinetz, Office of Science Policy (OSP), NIH

NIH/OD/OSP/BBEBP Staff Members

Shayla Beckham
Linda Gargiulo
Morad Hassani
Robert Jambou
Chengyuan Li
Maureen Montgomery
Marina OReilly
Eugene Rosenthal

[1] The Recombinant DNA Advisory Committee is advisory to NIH, and its recommendations should not be considered final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

Aparna Singh

Attendees

There were 60 attendees at this 2-day RAC meeting.

Attachments

Attachment I contains a list of RAC members, nonvoting agency and liaison representatives, and attendees present for the bioethics discussions. Attachment II contains a list of public attendees. Attachment III contains a list of abbreviations and acronyms used in this document.

I. Call to Order and Opening Remarks

Dr. Whitley, the Acting RAC Chair, called the meeting to order at 8:30 a.m. on June 21, 2016. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* was published in the *Federal Register* on May 27, 2016 (81 FR 33680). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of five gene transfer protocols, public review of updates in manufacturing of CTL019, and presentation of certificates of appreciation to retiring RAC members.

RAC members introduced themselves by name, affiliation, and research interests.

Dr. Tucker reminded RAC members of the rules of conduct that apply to them as Special Government Employees, read into the record the conflict-of-interest statement, and suggested that related questions be addressed to the OSP Committee Management Officer.

II. Minutes of RAC Meeting, March 8–9, 2016

RAC Reviewers: Drs. Hearing and McCarty

Drs. Hearing and McCarty found the minutes to accurately reflect the Committee's business at the March 8–9, 2016 RAC meeting. Both reviewers recommended approval of the minutes as written. No additional comments or changes to the minutes were suggested by other RAC members.

A. Committee Motion 1

Dr. Whitley asked the RAC to approve the minutes of the March 8–9, 2016, RAC meeting. The RAC voted unanimously by voice to do so.

III. Review and Discussion of Human Gene Transfer Protocol #1601-1502: A Phase I Safety Study of Adoptive Cellular Therapy Using Autologous T Cells Transduced with Lentivirus to Express a CD33 Specific Chimeric Antigen Receptor in Patients with Relapsed or Refractory CD33-Positive Acute Myeloid Leukemia

Presenters: William Wierda, M.D., MD Anderson Cancer Center
Mike Rytting, M.D., Children's Cancer Hospital at the University of Texas *(via teleconference)*

RAC Reviewers: Drs. Pilewski, Ross, and Whitley

A. Protocol Summary

Acute myelogenous leukemia (AML) is the most common form of acute leukemia in adults, affecting approximately 12,000 individuals in the United States per year. Most patients are diagnosed later in adulthood. The 5-year survival is approximately 20 percent; patients over the age of 65 have a particularly poor prognosis, with median survival less than 4 months. Most patients achieve remission with induction chemotherapy, but the vast majority subsequently relapse. A number of therapeutic approaches are under development, including alternative chemotherapy and biologicals. One such alternative, conjugated antibodies to CD33, has shown some promise. The first humanized anti-CD33 molecule approved by the FDA was conjugated with calicheamicin (gemtuzumab) after studies demonstrated reasonable safety and improved survival; the product was later withdrawn from the market, however, owing to lack of proven efficacy and significant dose-related toxicity in post-marketing studies. Given the poor prognosis and lack of effective therapy for patients with refractory or relapsed disease, many of whom are not candidates for potentially curative allogeneic stem cell transplant, new therapies for AML are necessary.

The proposed protocol is a single-center, proof-of-concept, first-in-human gene therapy study to evaluate the safety, feasibility, and persistence of adoptively transferred CD33-specific autologous T cells in research subjects with relapsed or refractory CD33-positive AML. This open-label Phase I trial will also determine the recommended Phase II dose of a single infusion of these CD33 chimeric antigen receptor (CAR) T cells for adult and pediatric patients as separate cohorts. The T cells are modified to express a second-generation CD33-targeted CAR with CD137 and CD3 endodomains and a truncated human epidermal growth factor receptor 1(HER1t). The *CD33-CAR* and *HER1t* genes are introduced into the T cells by transduction with a LV-CD33 CAR lentivirus. The dose escalation and Phase II dose determination will be conducted separately and in parallel for the adult and pediatric patients based on the standard 3+3 design. The dose levels, in increasing order, are 3×10^5 CAR+ cells/kg, 1×10^6 CAR+ cells/kg, and 5×10^6 CAR+ cells/kg. A dose level of 1×10^5 CAR+ cells/kg will be employed if de-escalation is required after dose level 1. The first dose cohort will be cleared with full safety monitoring and dose-limiting toxicity (DLT) assessment in adults prior to initiation of enrollment to the pediatric cohort (i.e., defined as younger than age 18). Pediatric and adult cohorts will subsequently enroll and be assessed in parallel. Dose-level cohorts will be enrolled sequentially. A dose de-escalation plan is in place if needed. Up to 18 adult and 18 pediatric patients will be enrolled.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because it is a first-in-human gene therapy trial to evaluate the safety, feasibility, and persistence of autologous T cells that are modified using a lentiviral vector to express a second generation CD33-targeted CAR to individuals with relapsed or refractory CD33-positive AML.

Three RAC members provided written reviews of this proposed Phase I trial. They considered the protocol to be well written and thoughtful and the research to be important. Dr. Ross found the protocol, answers to Appendix M, and the consent forms easy to read. The proposed regimen involving lymphodepletion followed by adaptive cellular therapy focused on CD33 is sufficiently different than treatment with only gemtuzumab ozogamicin, the previously FDA-approved anti-CD33 monoclonal antibody (mAb), which was shown to be ineffective.

Dr. Pilewski had the following comments and questions regarding the protocol and Appendix M:

- The protocol allows for bridging chemotherapy after leukapheresis and during production of the CD33 CAR T cells at the discretion of the treating physician. He acknowledged that there is no standard salvage chemotherapy for AML, but questioned whether variability of chemotherapy by the treating physician could affect subsequent eligibility for dosing and response to therapy. If so, why isn't bridging chemotherapy standardized to minimize the risk and confounding of the safety and efficacy outcomes?
- Given that expression of CD33 appears to be variable and not an all-or-none phenomenon, will there be a threshold for CD33 expression to meet inclusion criterion #3, "CD33 positivity"?

- In the studies of gemtuzumab, response rates appeared to be significantly affected by cytogenetic markers of relapse risk, with patients having favorable or intermediate risk deriving a relapse-free survival benefit (as reviewed in the meta-analysis from Hills et al., *Lancet Oncol* 2014). Will the proposed study, which is a Phase I dose-escalation safety trial, include cytogenetic markers as part of the safety and efficacy analysis?
- Liver toxicity, particularly veno-occlusive disease, is a known risk for gemtuzumab. In some clinical trials, the inclusion criteria required bilirubin and transaminases less than 1.5 times the upper limit of normal (ULN). In the proposed study, in which CD33 targeting may be more robust and confer higher risk, further information is needed to explain why inclusion criteria #6 and #7 permit bilirubin up to 3.0 mg/dL and transaminases up to 5 times ULN.
- One risk of CAR T cells is respiratory distress associated with capillary leak or cytokine release syndrome (CRS). The inclusion/exclusion criteria for underlying pulmonary disease are quite liberal insofar as short of mechanical ventilation, hypoxemia, and poorly controlled pulmonary disease, patients are eligible. The investigators should consider including more stringent criteria, such as exclusion of patients with advanced emphysema or interstitial lung disease or Class III or IV status to mitigate risk of death during the protocol.
- The cardiac arrhythmias that would exclude participation should be more clearly defined (exclusion criterion #5).
- According to the protocol, to be eligible for lymphodepletion with high-dose cyclophosphamide/fludarabine (CY/FLU), and for infusion of CAR T cells, serum creatinine must be less than 2.5-fold higher than baseline. As such, patients could undergo these interventions with some level of acute renal failure. Further discussion of the rationale for this cut-off is needed. The investigators should consider reducing the threshold to 1.5-fold higher than baseline.
- The protocol states that CD33 CAR T cell infusion can occur up to 14 days after lymphodepletion, but the investigators indicate 2 days as the optimal or ideal timing. The rationale for this wide range is needed.
- In the table for stopping and re-starting T-cell infusion, resolution of hypoxemia should be added to the re-starting criteria for hypoxemia.
- The efficacy data in NOD-SCID mice harboring the MOML-13 cell line is impressive, with survival to 29 days with CD33 CAR T cells vs. 15 days in controls. In Appendix M, CD33 CAR T cell–treated animals are reported to have had "complete survival." As prevention of relapse is a major goal of this therapy, what is the duration of the effect in the mouse model? Moreover, would comparative studies in this model of CD33 CAR T cells with gemtuzumab be informative from efficacy and dose selection perspectives?
- Bone marrow suppression and hepatotoxicity have been the major issues with gemtuzumab, presumably from on-target, off-tumor effects. Do the CD33 CAR T cells react with other members of the Sialic acid-binding immunoglobulin-type lectins (Siglecs) superfamily, such as Siglecs expressed on macrophages or other tissue resident cells that would raise concerns for additional organ toxicity?

Dr. Pilewski had the following additional comments and suggestions regarding the informed consent document (ICD):

- The ICD should explicitly state that the primary goal of this Phase I study is to determine the safety and tolerability of increasing numbers of cells, with little to no expectation of benefit. The document currently says that in addition to assessing safety of the study drug, the purpose of the trial is "to learn if CD33-CAR-T can help to control CD33-positive AML," which overstates the potential for benefit to participants.
- Patients are informed that they will receive FDA-approved "standard combinations of chemotherapy." The intent of this chemotherapy, lymphodepletion and conditioning for the CD33 CAR T cells, is not indicated, potentially giving the impression that the chemotherapy is being given to treat the leukemia. The reason for the chemotherapy used under this protocol should be clarified.
- The statement, "You may also want to ask about uncommon side effects that have been observed in small numbers of patients but are not listed in this form," seems unnecessary.

- The listing of side effects by column is distracting. A more conventional format that is easier to follow should be considered.
- Medical jargon such as “organ failure” and “lung inflammation” should be simplified or defined in lay terms for clarity.
- With respect to withdrawal from the study, the ICD should state that after infusion of the study product, the cells cannot be taken back, that is, there is no withdrawal from the T-cell therapy after it is given.
- The potential benefit is currently stated as follows: “The T-cell infusion may help to control the disease. Future patients may benefit from what is learned in this study. There may be no benefits for you in this study.” The investigators should consider stating instead that the benefits of this intervention are unknown and that while the T-cell infusion may help control the leukemia, there likely will not be any direct medical benefit to the patient.
- Death should be explicitly mentioned as a possible outcome in the discussion of risks from the intervention.

Drs. Pilewski and Ross commented on the statement that participants are “mandated” to enroll in a long-term follow-up (LTFU) study. Dr. Ross pointed out that one cannot mandate follow-up because participants have the right to withdraw at any time. The information on what is required for LTFU is inconsistent between the protocol and consent documents. It is fair to explain that the cells may persist for years and so LTFU is strongly encouraged. Because participants’ prognosis is poor, however, the plan for extended follow-up should be modified to reflect anticipated survival, and the consent language in particular needs to take into account that patients may not survive to completion of this research study to be enrolled in follow-up.

Dr. Ross had the following additional comments regarding the 15-year follow-up study:

- The consent form says that the study lasts for 12 months and then explains that subjects will be asked to be followed because the FDA requires that participants in gene transfer trials are followed for at least 15 years. A statement such as, “The investigators are obligated to follow you for 15 years, and we will ask that you sign a separate consent at the time of the infusion,” could be added for further clarity.
- Alternatively, there is no reason to separate these consent forms. Language to consider using in a single consent document is, “After one year, we plan to keep you enrolled for long-term follow up for at least an additional 14 years.” Dr. Ross asked the investigators to explain why they plan to use two consent forms if they do not agree with this suggestion.

Dr. Ross had the following comments and questions:

- Per the protocol, the first dose cohort will be cleared with full safety monitoring and DLT assessment in adults (>18 years) prior to initiation of enrollment of pediatric subjects (<18 years). Further clarification is needed regarding the plan for dose de-escalation. For example, if there are two or more DLTs in the lowest adult dose, will de-escalation to the −1 dose occur and will enrollment to the pediatric cohort be delayed until after the −1 dose is completed and there is only one DLT at this dose?
- If no dose level can be found in which fewer than one in six adults has a DLT, it appears that no pediatric subjects will be enrolled. These provisions need to be confirmed and clearly stated.
- The consent states, “You and/or your insurance provider will be responsible for the costs of the chemotherapy, and any costs related to the chemotherapy, such as infusion.” Per this statement, it appears that some individuals will not be able to do the pre-treatment chemotherapy if they cannot pay for the preparatory chemotherapy. The investigators need to explain the rationale for this provision, if correct. Dr. Ross suggested an option to use a flat rate.
- Institutional conflicts of interest (ICOIs) are significant, and there is a very detailed plan to manage these COIs. Further information is needed as to whether this approach/plan has been used before and whether it has been deemed acceptable by the FDA. The justification for doing this study at MD Anderson needs to be clearly stated. Dr. Ross also asked whether it would be easier to conduct this trial at another institution to avoid the existing COIs.

- Concerns regarding COIs are made more acute with respect to emergencies, when a participant may be cared for by an individual with a significant conflict of interest. This poses problems if the family wants to refuse treatment or elects to withdraw from the study.
- Appendix D mentions the very important issue of tumor lysis as a sign that the study medicine may be working: "Thus, a balance may need to be struck between immunosuppression to relieve adverse reactions and maintain beneficial anti-tumor effects." This underscores why it is critical that the subject is not being cared for by an individual with a significant conflict of interest.
- Such an issue is made more acute again since the consent form states: "If you suffer injury as a direct result of taking part in this study, MD Anderson health providers will provide medical care. However, this medical care will be billed to your insurance provider or you in the ordinary manner." The consent later states that MD Anderson may cover direct injuries. "If you become injured or ill as a direct result of taking part in this study, the sponsor may pay for the treatment of the injury or illness. MD Anderson cannot determine at this time what you may be reimbursed for. A financial counselor will be made available to you after the injury or illness is reported." However, isn't it too late to have the financial discussion as to what is and is not covered after the injury?
- The consent form also says, "In the event, a non-MD Anderson ethicist is not available, an MD Anderson ethicist will contact you to assist with your questions and concerns." Dr. Ross inquired as to who will be paying the non-MD Anderson ethicist, whether this conflict will be disclosed, and the type of liability insurance the outside ethicist will be required to have. In addition, the consent says that subjects can contact the MD Anderson Institutional Review Board (IRB) with any concerns and that an outside IRB is in place because of the institutional COIs. These issues need to be clarified and made consistent, including who will direct the participants to the outside IRB.
- Even with an outside ethicist, outside IRB, and outside data and safety monitoring board (DSMB), it is not clear that these external human subject protection committees/individuals are sufficient to mitigate the institutional COIs and to adequately protect study participants.
- The consent clearly states that the participant can withdraw from the study at any time and what will happen with samples. The document also needs to explain that once the adoptive cellular therapy has been administered, it cannot be removed and that subjects can only withdraw from being followed since the therapy is complete. It is then appropriate to explain that sampling is needed to know whether the cells persist and to encourage ongoing sampling even if the participant refuses to participate in further research.
- A child's right to dissent and whether dissent will be respected need to be confirmed and clarified. The consent form states, "The participant dissented, but the participant's parent(s)/guardian felt that the intervention(s) or procedure(s) involved in the research provide the possibility of a direct benefit that is important to the health and/or well-being of the participant and is available only in the context of this research study." However, the assent form tells the child that he/she "[has] been told that I do not have to be in this study. If I decide not to be in this study, no one will be mad at me. I may quit at any time, but if I do, I may need to take a different treatment." It is reasonable in a Phase I study for a child to have the right to dissent. If the child does not have this right, the value of asking for assent is not clear when dissent will be ignored. The investigators can state instead that they will involve the child and explain what is going to happen. Dr. Ross noted that in some states, one only needs written assent if the research does not offer prospect of direct benefit. She was not convinced, however, that this rationale is sufficient to justify not getting assent in a Phase I study where the safety of the agent, or what is considered a safe dose, in children is not known. Therefore, the child's dissent should be determinative. The protocol states, "Assent is the child's affirmative agreement to participate in the research. Failure to object may not be interpreted to mean assent." The justification for overriding the refusal of a minor, particularly a teen age 13 to 17 years old who needs to provide written assent according to Texas law, is therefore not clear.
- The lay abstract should explain that additional studies will be necessary because this is a Phase I study.

The reviewers questioned why participants will not be compensated for travel costs and time for study visits, as has become standard for many clinical studies.

Dr. Whitley noted that adapting a dose from animal studies for administration to humans can be difficult and inquired about how the dose of CAR T cells was determined (e.g., what correlates were made)? In addition, he asked whether the dosage in a pre-pubertal child will be the same as the dose in a post-pubertal adolescent or whether a dose de-escalation study should be considered for the younger children who may participate in this trial?

Dr. Whitley noted that the investigators will appropriately identify adverse events (AEs) and use criteria to determine dosage modification. However, it is not clear that a Data Safety Monitoring Board or Committee (DSMB/C) will have access to the data or be involved in decisions regarding the interpretation of AEs. Is such a DSMB/C planned and, if not, why not?

The investigators state that approximately 3 percent of individuals given CD33 CAR T cells are expected to have Grade 3 or Grade 4 liver toxicity that typically occurs 10 days after T cell administration. Dr. Whitley asked whether patients should therefore be followed in the hospital for a period longer than 7 days, as currently planned?

Dr. Whitley agreed with the other reviewers that the protocol and consent should not overstate the potential benefit to participants. The proposed trial is a first-in-human study, and the volunteers are participating purely from a research purpose with no identified hope of deriving benefit. It is not clear whether harm that directly results from the administration of CD33 CAR T cells is the fiscal responsibility of the patient or of the institution. These points need further clarification.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed. They went through their comments and the investigators' responses to their queries and suggestions.
- Dr. Pilewski found the response to Dr. Whitley's comment regarding anticipated benefit to be very clear in informing patients that the investigators do not expect subjects to benefit from participation in this protocol given the natural history of the disease and that it is not known whether the patients will respond to the intervention. As a first-in-human Phase I trial, the likelihood of benefit is very small and saying in the informed consent document, as originally planned, that the T-cell infusion may help control the disease does not accurately portray the benefit assessment. Dr. Zoloth added that the statement at the top of the consent that patients are being asked to participate because they have a CD33-positive relapsed or refractory AML that needs treatment is misleading and sets up the document as being about a study that provides a therapeutic intervention, and thus needs to be removed.
- Several RAC members were surprised that there will be no compensation for travel costs and time, which is a departure from what is typically provided to participants in clinical trials. MD Anderson used to provide such compensation but changed its policy when reimbursement became difficult to manage and was determined to be too costly and time consuming from an administrative perspective. Dr. Pilewski pointed out that patients are making tremendous sacrifices to participate in this Phase I study, for which there is no potential for direct benefit and for which they will be incurring additional financial costs on top of the inconvenience, burden, and risks of being enrolled in the trial. This is clearly an institutional decision and the investigators and sponsor should consider changing the policy to offer this compensation in return for the patients' participation. It was noted that companies are available that work with research coordinators and subjects to provide this service to study sponsors and institutions.
- Dr. Ross asked a follow-up question to clarify study-related costs covered under the protocol versus those that the patient and/or the patient's insurance provider will be responsible for. It appears that the protocol does not cover the cost of bridging chemotherapy and related procedures (e.g., infusion, lymphodepletion). These are aspects of the trial, however.
- Dr. Zoloth urged the investigators to remove the statement in the consent that if a subject is harmed as a result of participation in the trial, the patient and/or their insurance company will be

responsible for any medical care provided. She pointed out that no insurance company will pay for research-related injuries incurred in Phase I clinical trials. The statement in the consent is therefore not true. The patient (and/or his or her family) takes on this additional financial risk if such costs are not covered.

- Dr. Zoloth agreed with the concerns raised regarding coverage of the costs of travel, lodging, and study procedures and strongly endorsed the recommendations that the sponsor and/or MD Anderson should pay for these expenses and aspects of the trial. She commented that patients come to MD Anderson for treatment in part because of the country's failed health care system but that the patients' situation should not be worsened by having to pay their own way to participate in a research study. Dr. Zoloth noted the irony of this premier cancer hospital testing experimental treatments in humans and animals but providing free housing and food only for the lab animals. She stated that the investigators and sponsor are making a deliberate moral choice to not provide coverage because the accounting and reimbursement process is considered unwieldy, not because no funds are available, and strongly urged MD Anderson, as a national leader in the field, to cover costs as discussed. Ways of minimizing costs should be explored, and alternative strategies should be identified so that MD Anderson, the sponsor, or another entity covers these costs so that patients do not have this additional burden.
- Dr. Ross revisited her concerns regarding the child's dissent. She pointed out that for a Phase 3 trial with a prospect of direct benefit, the parent or guardian's consent can override a child's dissent, and this is explained to the child. In this Phase I trial, however, the likelihood of benefit is small; in general, when research is not being done for the prospect of direct benefit, the child's dissent is considered determinative. However, the consent form has been revised to say if the child does not agree to be in this study but their parents or guardians and the study doctor think that the child should be in the study, the child may be asked to be in the study even if he or she does not agree. While the investigators' view that it is inappropriate to ask for assent because the child is being informed that they will be participating in a trial, as they have also pointed out, as per Federal regulations, assent is an active affirmation of agreement. The investigators have two choices. One is to not ask the child to assent, which would be against the Federal regulations; the second is to respect their dissent, which is appropriate for a Phase I study. Dr. Ross did not concur with the investigators' legal argument regarding parental consent and the child's dissent for participation in a research study. Parents are legally responsible for the child's clinical care, but this is research, and research is not a right. Further, if the protocol says that a child's refusal is determinative, the child is to be excluded regardless of what the parents say, which is what the investigators need to follow. Dr. Ross pointed out that this is the consensus among ethicists as to how this issue should be addressed. In addition, she expressed concern that obtaining an outside opinion if the child and parents disagree could put undue pressure on the child to agree to enroll in the study even when the child has said this is not what he/she wants. This is inappropriate and should be removed from the consent/assent process/plan. Per these points, the consent and assent documents therefore need to state clearly that if the child refuses to participate, their dissent will be respected, and no further steps will be taken to try to enroll the patient or to override the child's decision.
- Dr. Zoloth agreed with the points made by Dr. Ross regarding assent as affirmative and dissent as determinative in a Phase I trial. Children should not be pressured toward participating in a research study without direct benefit, particularly studies such as the proposed trial, which is very rigorous (i.e., serial bone marrow aspirations/biopsies are required for monitoring over a 1-year period). Dr. Zoloth noted that without clearly stating in the consent that assent is an affirmative action and that dissent will be respected, thereby following Federal regulations, the consent process and document are inadequate.
- Dr. Zoloth closed her comments by urging the investigators to rethink the entire structure for how MD Anderson conducts these trials with respect to coverage of travel/lodging costs, study procedures, and research-related injuries as noted by the reviewers and discussed during the meeting.
- Dr. Whitley requested additional information as to how the doses were selected and whether the specified dose range is too narrow to assess safety of the investigational product. He also asked whether the dosage will be adjusted for pediatric patients and if the dosage will be the same for pre- and post-pubertal children?

- Dr. Whitley asked if it is MD Anderson policy to use a DSMB for Phase I studies or if the judgment as to whether an event meets the criteria for a DLT is made solely by the investigators?
- Dr. Zoloth noted that some language in the consent document could be edited for clarity. For example, she suggested modifying the second paragraph under the “”Purpose of the study” section in the consent to specify that the goal of this clinical research study is to find the highest tolerable dose of genetically changed T Cells that can be given “*before they make you sick*” if the language is targeted to teenagers. The same language should be used if the document is meant for parents, but the consent should also refer to “your child” in all places if the parent or guardian is giving consent.
- Dr. Ross recommended changing the term “individuals who volunteer” to “research participants who volunteer” in the consent because former term is vague.

D. Investigator Response

1. Written Responses to RAC Reviews

The sponsor was identified as providing the written responses to the RAC reviewers’ comments.

The sponsor confirmed that if there are two or more DLTs in the lowest adult dose, the adults will be de-escalated to the −1 dose level, and no pediatric cohort will begin until after the −1 dose level is completed with one or no DLTs. If no dose level can be found in which fewer than one of six adults has a DLT, then no pediatric subject will be enrolled. The protocol will be amended to address these possibilities.

MD Anderson Cancer Center has an open and active LTFU protocol to monitor subjects who participated in gene transfer clinical trials (“Long-Term Follow-Up Study of Recipients of Gene Transfer Research Protocols” [Protocol 2006-0676]). This protocol provides additional details surrounding the LTFU activities and enrolls subjects from several gene transfer protocols. Having a single LTFU protocol allows investigators to close individual protocols once all subjects have been treated and have had safety and efficacy monitored for purposes of the primary protocol objectives. This avoids having multiple protocols open for 15 years, while still being able to follow subjects long term.

The protocol and consent will be amended to indicate that participation in the LTFU study is not mandatory but strongly encouraged. The consent already includes language to indicate that the investigators are obligated to follow the subject for 15 years and that subjects will be asked to sign a separate consent for the LTFU study.

The informed consent document will be revised to explain that the adoptive T cell therapy cannot be removed after administration and that withdrawal would only be a withdrawal from being followed, as the therapy is complete. The consent document will also be revised to encourage post-administration sampling for persistence even if the subject withdraws from other activities.

The financial responsibilities information is provided to patients as part of the informed consent process. If a subject’s insurance provider does not cover “standard of care” costs in the management of his or her disease, then the subject will not be enrolled in the study unless he or she agrees to pay these costs out of pocket. Chemotherapy is considered “standard of care” in the management of AML, as well as all physician visits, lab studies, transfusion support, antibiotics, and associated care. The following is the revised text that will be included in the consent form. It is the standard MD Anderson wording for financial responsibility: “Certain tests, procedures, and/or drugs that you may receive as part of this study may be without cost to you because they are for research purposes only. However, your insurance provider and/or you may be financially responsible for the cost of care and treatment of any complications resulting from the research tests, procedures, and/or drugs, including hospitalization, nausea, vomiting, low blood cell counts, and dehydration. Standard medical care that you receive under this research study will be billed to your insurance provider and/or you in the ordinary manner. Before taking part in this study, you may ask about which parts of the research-related care may be provided without charge, which costs

your insurance provider may pay for, and which costs may be your responsibility. You may ask that a financial counselor be made available to you to talk about the costs of this study."

MD Anderson follows the University of Texas Committee for the Protection of Human Subjects (CPHS) policy for research on children, which is based on Texas law governing consent to medical treatment for research. The policy reads, "Generally, the child's veto of participation is sufficient to make the child ineligible for inclusion in the study. However, the parent or guardian, with CPHS and physician approval, may override the veto if the intervention will benefit the child." The assent has been revised to explain that although a child's parent or guardian decides about the child's participation, the child has the right to agree or decline to enroll in the study and to change his or her mind after enrolling if they want to leave the study.

The investigators agree that bridging chemotherapy for AML is a necessary option for some patients to control their disease during the production period for their CD33 CAR T cells. Some patients have very proliferative disease and cannot go untreated for more than a few days before their cell counts become dangerously high. There is a spectrum of proliferative disease seen in AML. This trial is open to relapsed patients, and the investigators expect that there will be a spectrum of number and type of prior treatments. Choice of bridging chemotherapy should be left up to the treating physician, since their clinical judgment is important to select an individualized treatment that will keep the disease in control. The main issue will be if the patient achieves a remission with bridging chemotherapy, which will potentially impact the safety results of the trial if there are no leukemia cells for the CD33 CAR T cells to respond and react against. The study team therefore wishes to keep the choice of bridging chemotherapy open to the treating physician and not be prescriptive on this point. As long as there is leukemia present (>5 percent bone marrow blasts), the objective of the trial (to determine feasibility, tolerability, and safety) will be possible.

Expression of CD33 in AML is variable but present in most cases of AML. There is a spectrum for level of CD33 expression, but the level of sensitivity to detect surface expression with standard clinical flow cytometry is relatively low. Only a few surface molecules may be required to engage the CD33 CAR to trigger CAR T-cell killing. Inclusion criterion #3 has thus been modified to require 5 percent of leukemia blasts to be positive for CD33.

Gemtuzumab ozogamicin is a CD33 mAb-drug conjugate. It is a CD33 mAb linked to calicheamicin. Calicheamicin targets and damages DNA, and is responsible for the cytotoxic effects of gemtuzumab ozogamicin. An anti-leukemia effect has been observed with naked mAb. The CD33 mAb simply delivers the cytotoxic agent to the intended target, the leukemia cells. Leukemias with cytogenetic abnormalities, with the exception of certain core-binding factor leukemias, are known to be resistant to the cytotoxic effects of chemotherapy. This is also true for calicheamicin and is the reason why the response rates were lower in patients with cytogenetic abnormalities who were treated with gemtuzumab ozogamicin. The mechanism of CAR T-cell cytotoxicity is typically independent of cytogenetic features, as in the cases of acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). The investigators expect this to also be the case in AML. They will evaluate patients for cytogenetic abnormalities at enrollment to correlate with outcomes, which is standard of care for untreated and relapsed AML patients starting a new treatment. However, no cytogenetic abnormalities will be considered exclusionary for the protocol.

Hepatic veno-occlusive disease and sinusoidal obstructive syndrome are risks of treatment with gemtuzumab ozogamicin. With this drug, the liver is exposed to calicheamicin, which results in these clinical conditions. These risks can be exacerbated in the setting of conditioning for allogeneic stem cell transplant, as well as in combination with standard chemotherapy regimens. The hepatotoxic effects seen with gemtuzumab ozogamicin may be due to liver metabolism of unconjugated drug, leading in turn to calicheamicin-induced damage, liver infiltration by leukemic blasts, and nonspecific uptake of antibody-toxin conjugates by Kupffer cells. The hepatotoxic effects also may be due to CD33 expression by hepatocytes and/or Kupffer cells, in which case the effects would be considered an "on-target" toxicity . Another possibility is that the hepatotoxicity occurs through other receptor-ligand mediated interactions in the liver that have not been identified. Data indicate CD33 expression by Kupffer cells and hepatocytes in the normal liver. The CD33 CAR T cells do not deliver cytotoxic, DNA-damaging agents to their target but

are directly cytotoxic to CD33-positive cells. Preliminary data for another product under development (CD33-ADC, vadastuximab talirine) show no off-target toxicities, including hepatotoxicity. The proposed trial will include close monitoring for hepatotoxicity with the CD33 CAR T-cell therapy, and it will restrict eligibility to include only those patients with total bilirubin less than or equal to 2.0 mg/dL, alanine transaminase (ALT) less than or equal to 2 times ULN, before lymphodepletion or at initial enrollment.

The inclusion/exclusion criteria for underlying pulmonary disease have been revised to exclude those who require supplemental oxygen or mechanical ventilation and have an oxygen saturation by pulse oximetry of 94 percent or lower on room air. The cardiac arrhythmias that will be excluded include any tachyarrhythmia that is not rate-controlled and symptomatic bradycardia, as reflected in the revised protocol. Because lymphodepletion contains fludarabine, which is cleared by the kidneys, the eligibility for lymphodepletion and CD33 CAR T-cell infusion was changed to a serum creatinine less than 1.5 times ULN.

The investigators explained that there is no established optimal window for CD33 CAR T cell infusion after lymphodepletion and no established post-lymphodepletion time after which the effects of lymphodepletion are no longer present. Typically, chemotherapy regimens are given on 28-day courses, suggesting that infusions done 21 to 28 days after lymphodepletion might not be effective. The investigators speculate that there might still be a lymphodepletive effect 14 days after chemotherapy and therefore included this time point in the window. Their aim is to have a window that is reasonable and includes time for issues such as fever or infection that can delay infusion of the CD33 CAR T cells. With this approach, the team hopes to optimize the likelihood that a subject receives the CD33 CAR T cells when the effects of lymphodepletion are still present.

Regarding the lack of payment or provisions for travel and lodging, the investigators explained that the Department of Leukemia at the University of Texas/MD Anderson previously had compensation for pharmaceutical-sponsored studies as allowed and indicated by the sponsors. However, it became so burdensome to manage the administrative aspect of this effort that such provisions were eliminated from the department's studies. Specifically, it became too costly and time consuming from an administrative perspective to collect all receipts and reconcile reimbursements for travel and housing. The infrastructure to perform these tasks does not exist.

The dose of the CAR T cells was derived empirically based on work with CD19 CAR T cells. However, the starting dose and subsequent dose levels for the proposed study were reduced relative to those of similar CD19 studies because CD33 is a new target. The disease burden in AML is expected to be consistent with that of ALL in the CD19 CAR T cell studies.

Prior CAR T cell studies included children, adolescents, and adults. All patients were dosed on a per-kilogram basis. In those trials, there was no adjustment for pubertal status, as such adjustments were not needed. In addition, the density of CD19 in these trials did not seem to influence the efficacy of the T-cell therapy. These findings might be extrapolated to use of the CD33 CAR T-cell product.

A DMC is planned and will evaluate the safety and treatment status of all subjects. Responsibilities of the DMC will include but will not be limited to the review of the study documents, review of safety data, and risk-versus-benefit analysis. The DMC will also be responsible for recommendations concerning dose level modification, continuation, termination, or other modifications of the study based on the observed beneficial or adverse effects of the study. The DMC's responsibilities, meeting schedule, data to review, and other specifics will be outlined in a charter external to the study protocol. The protocol will be updated to indicate that a DMC will provide oversight.

The rationale for following patients in the hospital for 7 days is to monitor for early CRS. Early CRS in the CD19 CAR T-cell experience can be severe with capillary leak, hypotension, and hypoxemia requiring intensive care unit support. CRS in these cases can also be associated with altered mental status and seizure. CRS evolves rapidly and requires prompt interventions and management. Typically, subjects will have developed fever by day 7 if this is going to happen. The spectrum of CRS has not included severe hepatotoxicity or marked elevation of liver enzymes. Subjects will be monitored in the outpatient setting

with frequent lab draws after the 7-day inpatient observation period, if the subject has not experienced CRS up to that point.

The section in the ICD regarding anticipated benefit has been revised to read, “The benefits of receiving T cell infusions are unknown. The T cell infusion may help control the leukemia, but given the severity of your condition, there likely will not be a benefit.”

The investigators agreed with the other recommendations regarding the ICD and will modify the proposed consent document accordingly.

2. Responses to RAC Discussion Questions

The investigators provided a detailed description of MD Anderson’s financial investments and conflicts of interest in relation to this research. As noted in the written response to the reviewers’ comments, MD Anderson no longer has an equity interest in the study sponsors, Ziopharm and Intrexon. In late January 2016, the University of Texas Investment Management Company (UTIMCO) informed MD Anderson that all MD Anderson equity (stock) in Ziopharm and Intrexon had been liquidated. UTIMCO is a statutorily authorized external investment management body that holds and manages stocks owned by University of Texas institutions. MD Anderson had received this stock under its license agreement with these two companies, and it agreed to transfer certain research programs and research technology rights to Ziopharm, and grant Intrexon a nonroyalty-bearing license. MD Anderson implemented an institutional conflict of interest management plan (as submitted/presented to the RAC) to manage the ICOI arising from MD Anderson’s equity interest in Ziopharm and Intrexon in relation to the clinical trials involving the two companies. The ICOI management plan is required to remain in effect unless and until MD Anderson no longer has that institutional financial COI. MD Anderson will be decommissioning the ICOI management plan for studies that are ongoing under such a plan, and is not required to implement that plan for future studies involving Ziopharm and/or Intrexon. Any references and language pertaining to MD Anderson’s ICOI and management approaches will be removed from the informed consent document to be used for enrolling participants to this protocol.

MD Anderson’s only remaining financial relationship with Ziopharm is under a research and development agreement. MD Anderson will continue to receive research and development funding from Ziopharm for the term of the research and development agreement with Ziopharm. The research development agreement provides for continuation of ongoing programs pursuant to a development plan prepared by a joint steering committee. MD Anderson has no remaining financial relationship with Intrexon. Neither the study principal investigator (PI) nor any of the co-investigators in the Department of Leukemia or Department of Pediatrics have any financial interest in Ziopharm or Intrexon; therefore, there are no COIs to disclose.

Dr. Wierda noted that providing travel and lodging compensation has become a huge administrative effort for MD Anderson to manage. As part of the team's early work, pharmaceutical sponsors covered travel and housing reimbursement costs. Study nurses initially helped with this effort when coverage shifted from the sponsors to MD Anderson, but it ended up consuming more of the nurses' time than patient care and research work. Dr. Wierda explained that given the number of clinical trials conducted at MD Anderson, the institution does not have the infrastructure to support the accounting required to manage travel reimbursement and compensation for housing. He added that because the patients are very sick and need considerable support, most are local and therefore receive treatment locally, whether it is standard care or as part of a clinical trial. As a result, travel and housing costs are minimal.

Regarding other costs covered under the protocol and for which the patient and/or the insurance provider is responsible, Dr. Wierda noted that this aspect of the budgeting plan is not in place yet. Lympho-depletion in the proposed trial will include three doses of fludarabine and one dose of cyclophosphamide. In other CD19 CAR T trials, lymphodepletion is included as standard-of-care chemotherapy even though it is not done for therapeutic intent for those patients.

Dr. Rytting addressed questions about a child's assent and dissent and noted the challenge in how best to approach this issue. In many trials, children are asked to assent even though they cannot dissent. In these cases, if the child cannot dissent, the rationale for asking for their permission to participate in a study is not clear. In response to the reviewers' comments, the language for the proposed trial was changed to be more inclusive for children who give dissent by talking to the parents again and having the option to seek an outside opinion about whether or not the child can go against the parents' wishes. If, in the end, the child absolutely dissents, particularly if it is an older child, then that decision will be respected. Dr. Rytting raised concerns about potential legal problems with dissent given the parents' role and responsibility in making decisions for their child. Following further discussion, Dr. Rytting agreed that active dissent from the child will be respected and that in such cases, the child will not be enrolled.

Dr. Wierda explained that the doses are based on empirical evidence. The doses for the proposed study were derived empirically based on protocols involving CD19-directed CAR T cells in ALL patients. The starting dose and subsequent dose levels for the planned trial were reduced relative to those of similar CD19 studies, however, given that CD33 is a new target. The protocol includes a plan for dose de-escalation if needed based on results for the starting dose cohort. The investigators expect the disease burden in AML should be consistent with that of ALL. Dr. Rytting noted patients in other CAR T trials were dosed on a cells-per-kilogram basis with no further adjustments for children or pubertal status. Results from those studies indicate efficacy at the chosen doses, suggesting that dosing based on weight is reasonable.

The proposed study has several levels of safety monitoring and study oversight. Investigators will assess adverse events to determine whether the events are DLTs. A medical monitor will review results at the completion of each cohort before any dose adjustments are made, whether it involves dose escalation or de-escalation. In addition, MD Anderson has an IND office to oversee the conduct of its trials independent of the investigators, who do not hold INDs. Per this policy, the institution is set up as the study sponsor to monitor the overall protocol and to review study data and outcomes and determinations of individual investigators. Oversight by a DSMB is considered on an individual study basis for IND trials at MD Anderson because of how the protocols and the DSM process are set up.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Clinical and Trial Design

- Recognizing that this is a Phase I study, the costs of research procedures should be borne by the protocol, which would include lymphodepletion chemotherapy, as well as other study costs that are not considered standard of care.
- Consider covering the travel/housing costs for participants (e.g., using flat rates for travel/housing costs), as Phase I studies are not the fiscal responsibility of the participant. Though it may not be institutional policy to cover these costs, compensation of research participants in these studies for their participation is strongly encouraged.
- Consider developing specific dosage standards for children, as the adult dosage may not be appropriate.

Ethical, Legal, and Social Issues

- Clarify the informed consent document (ICD) in lay language to indicate that:
    - This study involves research and not treatment,

  - The participants will be treated by an investigator,
    ,
  - The child's dissent is determinative. Please refer to CFR 46.402b, which explicitly states that the child's assent must be an affirmative agreement.
- Eliminate the language regarding insurance coverage for harm or injury and clarify in the consent process who will be responsible for the cost of injury.
- Consider changing language in the consent form from "individuals who volunteer" to "research participants who volunteer."

G. Committee Motion 2

Dr. Whitley summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Whitley requested a vote, and the RAC approved these summarized recommendations by a vote of 12 in favor, 0 opposed, 1 abstention (Atkins), and 1 recusal (Lee)

IV. Review and Discussion of Human Gene Transfer Protocol #1604-1524: Phase I Trial of Autologous T Cells Engineered to Express NYESO-1 TCR and Gene Edited to Eliminate Endogenous TCR and PD-1

Presenters:	Edward Stadtmauer, M.D., University of Pennsylvania Carl June, M.D., University of Pennsylvania J. Joseph Melenhorst, Ph.D., University of Pennsylvania
Participant:	Barbara Vance, Ph.D., University of Pennsylvania
RAC Reviewers:	Drs. Atkins, Cannon, and Cho

A. Protocol Summary

Recent studies have shown safety and promising efficacy of adoptive transfer of T cells with transgenic T-cell receptors (TCRs) specific for NY-ESO-1 in myeloma, melanoma, and sarcoma. Studies also show, however, that in many instances, the gene-modified T cells become exhausted and may, in turn, cease to proliferate, lose function, or fail to persist. To overcome these deficiencies, the study team has engineered autologous T cells that express a TCR with specificity for NYESO-1 peptide in which the endogenous *TCR (TCR^{endo})* and programmed cell death protein-1 *(PDCD1* or *PD-1)* gene loci have been deleted by gene editing using an efficient clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) technology. Targeted gene modifications by CRISPR/Cas9 require the introduction of a Cas9 nuclease and guide RNA (gRNA) into cells of interest. The *PD-1* gene product (CD279) in particular is targeted because it has been identified as a T-cell exhaustion marker in cancer and HIV and may lead to reduced or defective memory T-cell formation.

This approach offers two potential benefits over previous work in that the recombinant NY-ESO-1 T cells (1) have higher expression of the transgenic TCR and do not have the potential for mispairing with the endogenous TCR, and (2) are resistant to the checkpoint ligands PDL1 and PDL2 that may be expressed by tumor cells or by cells in the tumor microenvironment. Given the poor prognosis with standard therapies in the proposed patient populations, the investigators hypothesize that checkpoint-resistant T cells may be safe and have improved antitumor activity.

The proposed research trial will investigate a first-in-human application of the CRISPR/Cas9 technology to determine whether this strategy will create "checkpoint" resistant T cells. The protocol also has the potential to advance the field by testing CRISPR/Cas9 technology. The trial is designed to test the safety of autologous T cells that have been genetically modified in four ways: (1) with a lentiviral vector to introduce a high-affinity TCR directed to NY-ESO-1, (2) using CRISPR/Cas9 editing to knock out the

alpha chain of the endogenous TCR, (3) using CRISPR/Cas9 editing to knock out the beta chain of the endogenous TCR, and (4) using CRISPR/Cas9 editing to knock out the checkpoint inhibitor, *PD-1*. The final multiple-modified cell product is sometimes abbreviated as NYCE (NY-ESO-1 CRISPR Edited) T cells. The rationale for editing the endogenous *TCR* is to reduce *TCR* mispairing and promote expression of the exogenous NY-ESO-1 TCR. Editing of *PD1* is intended to maintain their activity in the presence of ligands (e.g., PDL1 and PDL2) including those in the tumor. Because each of the four proposed genetic modifications is not 100 percent effective, the end result is expected to be a mixture of up to 16 different types of cells.

A single infusion of 1×10^8 cells/kg of the bulk-treated T cells will be given so that the intervention is a "competitive repopulation strategy" whereby the location, persistence, and bioactivity of the edited cells found in the blood and the tumors will provide information to address the hypothesis that disrupting PD-1 can lead to better performance of the NYCE T cells. Fifteen adult patients with refractory tumors of multiple myeloma, melanoma, or sarcoma that express NY-ESO-1 and are human leukocyte antigen (HLA) A0201+, to be complementary to the high-affinity TCR, will be enrolled. The primary endpoints are safety and feasibility. Secondary endpoints include the engraftment, persistence, trafficking, and bioactivity of redirected NY-ESO-1 and CRISPR-edited T cells and to determine whether the cells generate any immunogenicity.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because the study involves the first-in-human combination of immunotherapy (autologous T cells engineered to express NY-ESO-1 TCR) and gene editing using the CRISPR/Cas9 system.

Three RAC members provided written reviews of this proposed study.

Dr. Atkins considered the protocol to be very thorough in describing the possible risks and issues associated with this approach. He noted that there are extensive preliminary *in vitro* data supporting the ability of edited T cells to function, proliferate, and continue to express the NY-ESO-1 TCR.

Dr. Atkins had the following questions:

- The investigators state that the usual bone atrophy in the proposed model is predictable. However, in the introduction to the clinical protocol, they state that the response to current therapy in this model is variable.
- How will research participants be maintained for the 35 days that it takes to manufacture the cells? Will research participants with disease progression be replaced?
- How will enrollment be coordinated between the three study sites and three different disease populations?
- How will protocol agent be deactivated if excess toxicity is observed?
- How much gene editing is possible in the transfused T cells? Will it be 100 percent, and will it be consistent from research participant to research participant?
- Please explain how "off-targeted editing" will be identified and how this will be handled if it is noted.
- It does not appear that interleukin-2 (IL-2) will be given to maintain T-cell survival and function. Will this be considered in the future?
- Will immunogenicity of the deleted T cells be an issue? Does the deletion leave expression of an abnormal protein(s) on the T cell surface?
- What is known about tumor escape from NY-ESO-1 transduced T-cell infusions?

Dr. Cannon had the following comments and questions for the investigators:

- The proposed genetic engineering involves four different modifications, which can produce 16 different combinations of cell genotypes. The frequency of the ultimate desired cell product in the bulk-treated NYCE populations does not appear to be presented, however. Some of this

difficulty is because interpretation of the assays is not straightforward (e.g., fluorescence-activated cell sorting [FACS] analysis for CD3 will recognize both the endogenous and exogenous TCRs). In addition, the Surveyor assay data presented is labeled as percent gene knockout (KO); while technically correct, this assay is a measurement of all alleles in the cell population and therefore does not provide any information about the number of cells with the desired bi-allelic disruption that would create a phenotypic knockout. Finally, in some cases, absolute values of gene disruption are not available but are instead extrapolated from partner or control samples. Assays such as deep sequencing and clonal analyses would complement these studies and give a more definitive assessment of the frequency of the 16 different edited cell genotypes. It does not appear that such assays are included in the planned analyses. The investigators do state, however, that digital droplet polymerase chain reaction (ddPCR)–based assays are in development to measure the gene knockout frequencies of *PD-1* and *T cell receptor–α* (*TRAC*) and *–β* (*TRBC*).

- The data provided for the two T-cell donors shown seemed to show very different levels of some of the modifications. Given this information, what is the range of modification seen over a more extensive set of donors?
- Measuring the possibility for off-target editing for each of the three gRNAs is an important part of preclinical safety studies involving targeted nucleases. These analyses do not appear to be part of the proposed research, however. Dr. Cannon noted the following in Appendix M:
    - The statement, "All gRNAs contained more than 13 base pair (bp) mispairs to exclude potential off-target mRNA [messenger RNA] sites," does not provide sufficient detail for interpretation. Moreover, it seems to imply that lack of off-target activity is predicted rather than having been confirmed experimentally.
    - The appendix refers to methods to detect genetic modifications outside of the target RNA sequence as "under development." What is the status of these methods, and have the selected gRNAs been validated for their off-target profile?
    - Both the appendix and the clinical protocol state that "off-target effects … [are] a possibility" that the investigators need to consider and that "whether this will occur with our CRISPR/Cas9-edited cells will have to be investigated in the infusion products." Is there an assay now in place to do this, and how will the results of this analysis be included in the release criteria?
- Using CRISPR/Cas9 to simultaneously edit three different loci raises safety concerns beyond the normal concerns about off-target editing from a single gRNA. Specifically, was the risk of translocations occurring between two different DNA breaks assessed?
- Four different genetic modifications are being attempted. Is there a lower cut-off limit of modification to define success? For example, it is stated in Appendix M that lentiviral vector transduction must be greater than 2 percent, but there is no similar metric for each of the three different gene knockouts.
- In the Study Summary and Study Schema section "Study Product, Dose, Route, Regimen," the term "CRISPR RNA" is not correct to describe the active component; rather, it is a combination of Cas9 mRNA and three different gRNAs.
- It is not clear whether the Cas9 being used as the standard *Streptococcus pyogenes* protein or a different variant.

Dr. Cho noted the following regarding the anticipated risks of the study intervention:

- Dose-related toxicity of NY-ESO-1–directed TCRs has been tested in several clinical studies, including Phase II trials conducted by the current research team. These studies have demonstrated safe dose ranges of up to 1.30×10^{11} cells, with favorable response rates. However, toxicity of additionally including cells with deactivated PD-1 is unknown.
- Regarding on-target reactivity, in two trials, 29 of 36 total research participants developed erythematous skin rashes that were thought to represent on-target reactions to antigen on normal cells.
- Although uncontrolled T-cell proliferation has not been observed by the investigators, the investigators plan to use corticosteroids and chemotherapy if necessary. Transformation of T cells in adoptive T-cell transfer has not been observed in hundreds of research participants

receiving retroviral modified cells or in 21 HIV research participants receiving lentiviral modified cells.
- Immunogenicity of CRISPR/Cas9-modified T cells is unknown and will be monitored as a secondary objective.
- In contrast to CAR-redirected T-cell treatments, CRS did not occur in research participants in studies testing NY-ESO-1 TCR-redirected T cells, despite elevated levels of IL-6. However, the investigators state that the NY-ESO-1–redirected T cells with edited TCR and PD-1 may induce CRS and that treatment of the CRS (e.g., with anti–IL-6 receptor antibody) could adversely affect the anti-tumor response.
- Risks for tumor lysis syndrome (TLS) are typically dependent on disease and burden of disease, but the investigators have a plan to monitor and manage TLS as needed.
- The investigators believe risks for autoimmunity to be low, but they state that there is potential for enhanced risk from transfer of T cells that have lost PD-1 expression but retained endogenous TCR expression.
- The risks of generating replication-competent lentivirus (RCL) have been minimized through design of the vector, by excluding individuals with HIV infections, and by monitoring for RCL for up to 2 years after infusion.

Dr. Cho requested the following additional information to better understand the potential risks of the study:
- What are the results of assays that were developed to detect genomic editing by CRISPR/Cas9?
- Are there any results of animal studies that assessed the safety of triple-editing similar to the proposed Phase I trial using NY-ESO-1?
- Provide a plan to assess off-target effects.

Dr. Cho identified several additional sections and statements in the consent document that should be clarified or revised. The section about the purpose of the study should state clearly that the purpose of the study is to test a variation of immunotherapies that have been tested in humans but that the variant that will be used in this trial has not. Statements about withdrawing from the study should be clarified to say that it might be impossible to reverse the effects of the infusion. In the discussion of alternatives to this trial, it should be made clear that participating in this study is not the only option for patients to obtain immunotherapy. It is not clear why Social Security numbers are necessary for the research. The section about use of blood and tissue should clarify whether research participant samples will retain any linkages to identifiers even if identifiers are removed before being given to other researchers, and whether withdrawing from the study also means withdrawing from studies being conducted by other researchers who obtained the samples. Use of the word “cure” is potentially coercive because a “cure” for cancer in general is implausible.

She noted that Dr. June is the scientific advisor for the study and, as disclosed in the consent document, has a “significant” financial interest as inventor of the technology used in the study, which is licensed to Life Technologies and sub-licensed to Novartis. However, no further detail regarding this COI is provided, and no mitigation strategies are described. The magnitude and nature of the COI should be specified in the protocol and consent

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:
- During the 35 days that it takes to manufacture the T cells, patients will be allowed to receive standard therapies up to two weeks before administration of the experimental intervention; if the manufacturing of the cells fails, or the patient's condition deteriorates during this time, the subject will no longer be eligible and will be replaced. Dr. Atkins requested additional information as to what will happen if the patient improves on the interim standard treatment, and whether the engineered cells can be kept frozen for future use should the patient subsequently become eligible for the intervention.

- Dr. Atkins noted that it might be difficult to identify sufficient numbers of melanoma patients who meet all inclusion criteria, specifically, those who are HLAA2- and NY-ESO-positive, who have received four prior therapies, have biopsy-accessible disease, and are otherwise healthy enough for this protocol. He applauded the investigators' efforts to recruit this population but commented that they might need to accrue more myeloma and sarcoma research participants if enrollment of melanoma research participants falls short.
- Dr. Atkins found the plan to deactivate the study agents and, if necessary, eliminate the engineered T cells if excess toxicity is observed to be appropriate and clearly delineated for each potential toxicity that could occur. He noted further that the University of Pennsylvania (UPenn) is a leader in this field for managing and reducing risks of CRS and autoimmune responses.
- The degree of editing is expected to differ among research participants, as previously demonstrated. For example, 20–55 percent loss of PD-1 has been shown with the proposed CRISPR system, while other data suggest an 11–28 percent loss of chemokine receptor type 5 (CCR5) using a zinc finger nuclease (ZFN) editing technique. Dr. Atkins commented that it is important to point out that the goals of this research include demonstrating the feasibility of the CRISPR system and identifying the resulting products.
- Dr. Atkins commented on the promise of this gene editing approach and the knowledge that is expected to be gained from the proposed trial, which, in turn, could form the basis for new types of therapy. While there are concerns regarding the patient population and potential toxicities, these issues appear to be addressed so that research participant safety is maximized and risk is minimized.
- Dr. Cannon acknowledged that this protocol submission is not an IND application and that it is not the role of the RAC to evaluate INDs, but that some of the information and responses provided by the investigators will be included in an IND submission (e.g., expanded analysis of more donors). She noted further that the investigators have clarified that the proposed study is a Phase I safety and feasibility trial; revisions to the protocol will reflect this.
- The investigators were encouraged to explore more sensitive assays to capture any potential genomic rearrangements. Off-target activity is a novel aspect of this research involving first-in-human application of multiplex CRISPR.
- There is now extensive clinical experience with first-generation trials of NY-ESO-1 TCR only (i.e., without the additional modification of the NY-ESO-1 CRISPR edited T cells). Dr. Cannon noted that based on results of these trials, there is a reasonable expectation of efficacy from just doing the lentiviral vector transduction. She asked if there is any concern that the additional modifications could reduce the efficacy of the NY-ESO-1 TCR and the final T cell product. Further confounding this concern is the plan to use a 10-fold lower dose of NYCE T cells than that used in the NY-ESO-1 TCR trials. While it makes sense to be cautious and lower the dose for safety reasons for a novel product being tested in a gene editing protocol, the impact on efficacy of the T cells is not clear. In addition, based on data from the presentation, it appears that there may be fewer PD-1-disrupted (KO) cells than anticipated given reduced survival of these cells during T cell contraction. The combination of the lower dose and fewer PD-1 KO cells could further exacerbate the efficacy problem.
- Dr. Cannon noted that the investigators had achieved low levels of toxicity in combination with reasonably high levels of gene editing of three loci. The study hypothesis will be tested using a competitive repopulation assay to determine whether knocking out the endogenous TCR gene will produce an enhanced survival effect in wild-type PD-1 cells versus the PD-1 knockout cells and whether one cell population is effective early or later compared with the other cell population. It is likely that this Phase I study will generate some interesting biology.
- Dr. Cho asked whether the assays being developed to detect genomic editing by CRISPR are able to identify triple-edited cells, or if they are designed to detect each target individually. She also requested additional information about the many cells have gotten three hits and the proportion of the overall cell population that has been triple edited (in both animal and clinical studies).
- Dr. Hearing requested additional information as how a clonal population of cells that grew out in a research participant would be analyzed, including whether such analysis would include whole-genome sequencing.

- Dr. Cho noted that the consent needs to include a statement that is more explicit regarding the meaning of withdrawal from the study, specifically, that once the investigational product is infused, it cannot be removed and that there is no guarantee that any side effects of the product can be reversed. Thus, withdrawal refers to no longer being followed under the protocol.
- Dr. Zoloth requested clarification as to the plan to have research participants enroll in a long-term follow-up study once they have completed the original two-year trial in which they are administered the NYCE T cells. The two protocols have different designs, and given that the proposed study involves first-in-human use of CRISPR, it is not clear why research participants will be moved to another protocol instead of staying in the original trial for ongoing monitoring and follow-up. Dr. Zoloth noted further that the consent should clearly explain the significant difference in how cells are modified using CRISPR compared with other procedures and that because this system has not been previously tested in humans, there are concerns as to potential unknown risks.
- Dr. Cho followed up on disclosure of Dr. June's COI in the consent. Regardless of, or in addition to, what the local IRB requires (or doesn't), the ICD should not only identify any financial and other COI, the document should include a description of what the COI means. Dr. Cho also requested additional information regarding Dr. June's role as scientific advisor on the protocol given his significant financial COI. Specifically, any involvement in patient interactions, study design, data analysis, and other activities should be described, along with plans on how this COI will be mitigated. Along similar lines, Dr. Ross asked whether the other sites or investigators have any financial COIs. She expressed concern that UPenn is serving as the lead institution for the proposed protocol rather than one of the other institutions that do not have any known COIs.
- The investigators were asked to walk through the relationship between Novartis, Parker Institute, and Life Technologies, which appear to reflect three different potential financial COIs. In addition, clarification was requested regarding the relationship between Parker Institute, which is funding the trial, and the three participating institutions (UCSF, MD Anderson, UPenn).
- Dr. Donahue considered the issues raised about UPenn's COIs to be valid, but he did not agree with the suggestions to limit the institution's activities solely because of these conflicts. He referred to HHS guidelines, which say nothing in support of that level of restriction. All COIs need to be disclosed, and a plan to manage these COIs needs to be in place. A recommendation from the RAC to exclude certain activities seems to be beyond what is in stated policy. Dr. Ross commented that COIs represent a real problem and have resulted in bad outcomes in the past. Having the other study sites be responsible for subject recruitment would likely engender greater trust in the broader community. Dr. Zoloth added that this issue needs additional reflection on the part of the Principal Investigators. She and Dr Wooley noted not only UPenn's current COI but its history of substantial financial COIs to explain why the RAC has engaged in an extensive discussion of the issue for the current trial and has considered limiting some of the institution's activities. Dr. Cho added that while these COIs may not lead directly to specific decisions or outcomes, the associations exist and there is at the very least the perception that the COIs could influence major aspects of the study. Recusing certain individuals from specific tasks, and having truly independent review and oversight are ways to mitigate these COIs The extent to which these COIs can be managed is critical, but given the collaborative nature of the proposed research and UPenn's history, the study remains somewhat vulnerable to these issues and to criticisms as to how they are handled.
- Dr. Ross expressed concern about subjects' having to cover the costs of standard medical tests, exams, and procedures that are required under this research protocol. These exams and procedures are part of routine cancer care and are done over the course of the trial. Subjects who find out that routine cancer care is not being paid for may choose not to have those tests and exams, thereby impacting the study. The subjects have already received the CAR T cells and want to otherwise participate but do not see the value of the additional costs to them. In those cases in particular, it is not clear why the trial does not pay for those extra cancer tests. The study also team needs to keep in mind that the potential financial success of this intervention depends on the integrity of the study. If participants do not have those tests because the tests are not "part of the research," the data will be incomplete. While the patient's insurance company should cover the cost of clinical tests, individual subjects should not be liable for these costs. Drs. Ross and Zoloth also expressed concern that if subjects end up having to pay for costs of additional routine

medical tests, the investigators potentially risk excluding an entire subgroup of this patient, namely, poor individuals and those without insurance.

- During the meeting, Dr. June clarified that research participants will not be responsible for the cost of standard-of-care medical tests and procedures, or for any investigational aspects of the protocol. In response, Dr. Ross pointed out that the consent document needs to be revised to reflect what procedures are covered and who will cover those costs. The ICD currently states that taking part in this research study may or may not cost the research participant and/or their insurance company more than the cost of getting routine. The document says that the research participant is expected to pay for any costs not paid by their insurance provider, including co-pays and deductibles. Dr. Zoloth added that the protocol and consent in particular need to clearly state that research participants will not be receiving any clinical care or treatment in the proposed trial or in any other Phase I research study. In addition, the consent needs to clarify that it is very unlikely that the participant will receive any benefit from participating in this early-phase research protocol.
- Dr. Ross also noted that the travel and lodging costs will not be covered. She inquired about accommodations for research participants and their families, who will need to remain close to the study site for a certain period of time for research participants to complete both research and non-research aspects of the protocol.
- In continuing discussion of these issues, Drs. Ross and Zoloth pointed to the trend across many research studies to cover fewer and fewer aspects of a trial, even though these components are required to participate. The lack of coverage of travel and lodging expenses adds research participants' burden, especially when the protocol involves repeated in-person visits. Research participants make significant sacrifices in volunteering to participate in these research studies, which should be structured to assure that research participants are properly taken care of and treated, including providing for housing, travel, and other incidentals. Poor and middle-class individuals and families should not be excluded for financial reasons, and such provisions need to be re-assessed where they are in place or under consideration. Dr. Ross stated her concern about the lack of protections in place for these groups. Dr. Zoloth noted that how a trial is structured is a moral choice. Efforts should be made to adjust the budget and identify alternatives so that subjects are treated with respect and dignity.
- Dr. Zoloth suggested using the word "investigator" instead of "study doctor" in the consent to better convey the nature of the relationship in a research trial as between the investigator and subject, not between a patient and the study doctor.
- The investigators may want to revise the exclusion criterion for patients with "active HPV, HCV, or HIV infection," which can be defined in different ways. They may want to change this to evidence of infection detected by PCR of RNA or DNA.

D. Investigator Response

1. Written Responses to RAC Reviews

Patients will be allowed to receive standard therapies to maintain disease control after apheresis and up to two weeks prior to infusion, as described in the protocol. Patients with disease progression (or cell manufacturing failure) will be replaced. The patients with cell manufacturing failure will be included in the analysis of feasibility endpoint.

The current plan is to open the University of Pennsylvania site first, followed by opening the other sites at the University of California, San Francisco (UCSF), and MD Anderson Cancer Center (MDACC). Enrollment will commence at the UPenn site and continue with the other sites in the order in which they are activated. Each site will take turns enrolling the research participants. The first research participants will be those with multiple myeloma as this patient population has been previously tested for safety of NY-ESO-1 TCR-redirected T cells at UPenn. Myeloma research participants will be enrolled at UPenn and UCSF, melanoma research participants will be enrolled at MDACC, and sarcoma research participants will be enrolled at MDACC and UPenn. Research participant enrollment will be coordinated by a dedicated project manager within the Regulatory Sponsor team at UPenn, ensuring balanced allocation at the three sites and coordination with the UPenn Clinical Cell and Vaccine Production Facility (CVPF).

Management of toxicity, including strategies to inactivate modified cells, is described in the clinical protocol. Inactivation strategies are considered for uncontrolled T-cell proliferation, tumor lysis syndrome, autoimmunity, and cytokine release syndrome. In cases of uncontrolled Tcell proliferation, corticosteroids and chemotherapy (cyclophosphamide) would be given to eradicate the modified cells. This strategy has worked in previously published studies. Campath is also considered in these situations. Tumor lysis syndrome will be managed with intravenous (IV) fluids and rasburicase as needed and additional clinical therapy as appropriate; prophylactic administration of allopurinol is at the discretion of the clinical investigator. Autoimmunity is a potential effect of this investigational therapy and will be managed according to the standard-of-care principles that are evolving for immune-oncology. Depending on the toxicity severity, the clinical investigator may choose to eradicate the modified T cells using the agents mentioned above, in consultation with the rheumatology service. For CRS, anti-cytokine therapy, steroids, and supportive care will be considered. The UPenn team has developed an algorithm for CRS clinical management that includes strategies to block cytokine release based on the team's extensive experience with CART19-related CRS management. The algorithm will be included in the clinical protocol for guidance.

The frequency of NY-ESO-1 TCR lentiviral transduction and the Cas9 mRNA and gRNA electroporation efficiencies will vary among donors and cancer patients. This variation will be larger in various cancer histotypes, given the differing impact of chemotherapy regimens on lymphocytes. The team plans to expand the analysis using a larger range of donors to better define the potency and limits of the system and to define realistic criteria for clinical products release. One of the primary endpoints of this study is testing the manufacturing feasibility, and this expanded analysis will be included in the Investigational New Drug (IND) application.

For this protocol, research participants will be closely monitored following the infusion of the NYCE cells using deep sequencing, quantitative PCR, and ddPCR. Cases with unusual kinetics of the infused cells (e.g., patients with secondary expansions of gene-modified cells) will be examined in detail for clonal T-cell abnormalities.

The investigators pointed out that all gene editing strategies developed so far have some nonzero rate of off-target editing. For example, several off-target sites were found in the team's preclinical studies with ZFN–mediated editing of CCR5. However, in up to six years of ongoing follow-up of research participants in the corresponding protocol trial, there have been no clinical consequences due to off-target editing. The proposed trial is designed to assess for off-target editing as a primary safety endpoint. Research participants will be followed per FDA guidelines for 15 years to gather clinical and laboratory information on the potential consequences of off-target editing.

For laboratory studies, the "gold standard assay" for off-target editing is the long-term culture of T cells to assess for transformation in culture. In the assay in which off-target modification of *ANK1* was identified, the cell population in which this off-target deletion occurred was subjected to a long-term expansion culture to assess for potential transformation events, in parallel with other triple-gene modified and nonmodified control cell populations. All cultures showed similar growth curves over the entire period tested and thus excluded any gene editing–associated transforming events. These data will be submitted to FDA for review.

The team's laboratory investigations will be guided by clinical observations. Clonal T-cell expansions in a patient will trigger a detailed investigation to exclude off-target editing. Such incidences will be reported to the FDA and the RAC, and a subsequent investigation would be conducted in consultation with these groups. The investigators do not plan to assess every cell product prospectively for off-targets effects, but they propose testing the clinical cell products in long-term culture conditions for potential transforming events as a retrospective analysis, not as a release criterion. In the previous study with ZFN-modified T cells, a pentamer assay and surveyor nuclease assay were used to monitor patient products and samples post-infusion for on-target editing. For the proposed study, the final release criteria for the manufactured products will be defined in collaboration with the FDA.

The investigators do not plan to infuse IL-2 in the research participants following T-cell infusion. In the recent study with NY-ESO-1 T-cell infusions that were given without IL-2 support, many research participants had long-term engraftment persisting for at least a year. To maintain comparability between that trial, which did not employ CRISPR editing, and the present trial, the investigators want to avoid the addition of other variables such as IL-2 infusions. In addition, research participant safety assessments are more straightforward in the absence of the background of toxicity that would be expected from IL-2. Finally, a comparison of CAR T-cell infusions in research participants with and without IL-2 support did not indicate enhanced CAR engraftment in the cohort given IL-2.

It is possible that gene editing may induce synthesis of abnormal proteins and new epitopes and that these may be immunogenic. Immunologic elimination of gene-modified T cells has not had clinical consequences (i.e., it has been clinically silent) in a number of previous studies documenting lack of persistence for genetically modified T cells. This was a theoretic risk in the previous study with ZFN-modified T cells, and to date, there has been no evidence for immune reactivity to the modified T cells because the T cells have persisted long term in the research participants. It is possible that tolerance to PD-1 or the TCR locus could be altered by the infused products, and in such cases investigations will be guided by the specifics of the clinical presentation. Most likely, any immunogenicity will be clinically silent and recognized by the rapid disappearance of the infused cell product. The investigators noted further that Cas9 is a protein of bacterial origin and may be immunogenic. They are currently investigating the decay rate of the Cas9 at the mRNA and protein levels and also intend to test Cas9-specific immunogenicity in patient samples. The proposed study is designed to determine the immunogenicity of the investigational cell product as a secondary objective.

Prior studies have shown that some patients with myeloma whose tumor was targeted using NY-ESO-1–specific TCR-transduced T cells develop antigen escape variants. This does not appear to be the case in synovial cell sarcoma, however, where research participants maintain homogenous expression of NY-ESO-1, even when progressing after NY-ESO-1 T-cell infusions. NY-ESO-1 has been chosen as a target for the proposed trial because of the extensive clinical experience with this target. The investigators consider it a “tool.” Should CRISPR editing prove safe as an approach to decrease checkpoint resistance using this tool, then future trials would seek other targets with additional TCRs or CARs and PD-1 disruption. NY-ESO-1 TCR gene transfer and gene disruption efficiencies will be assessed at the single-cell level in manufactured cells to better understand gene engineering technology. These assays will complement ddPCR assays.

The final lower cut-off limit for gene editing has not been fully defined yet due to the limited number of donors tested thus far in preclinical studies. Plans to expand this analysis with additional donors and to perform small- and large-scale validation experiments in good manufacturing practice (GMP) conditions will be used to define the gene editing release specification based on experimental data. Based on previous data with CART19 T cells, the team can monitor and observe clinical effects when a population of T cells is infused with as little as 2 percent to 3 percent modification. For the ongoing CCR5 ZFN protocol, the lower cut-off is “CCR5 genome modification present” by MiSeq analysis. A high bar is not required for Phase I testing where safety and feasibility are primary endpoints. For potential efficacy testing in Phase II trials, a higher disruption frequency will be set based on the data from the Phase I trial.

The investigators agree that “CRISPR RNA” is not the correct term to describe the active component. Rather, the active component for the proposed study is a heterogeneous population of autologous T cells expressing NY-ESO-1 TCR with *TRAC, TRBC,* and *PD1* gene disruptions. This investigational cell product is manufactured by treating T cells with an NY-ESO-1 TCR-encoding lentiviral vector and a combination of Cas9 mRNA and three different gRNAs specific for targeting *TRAC, TRBC,* and *PD1* genes.

The Cas9 being used is the standard *S. pyogenes* protein used for CRISPR/Cas9 systems.

The investigators have addressed the other recommendations regarding the ICD and submitted a revised document that tracks the changes made.

2. Responses to RAC Discussion Questions

The investigators tested the efficacy of triple editing CRISPR technology in two donors. For each of these donors, there were three conditions in which titrating amounts of gRNA were used (low, medium, and high gRNA levels for a total of six gene-edited samples and two nonedited samples). Initial preclinical data indicate that the proposed CRISPR system induces PD-1 gene editing in 20 percent to 55 percent of alleles depending on the amount of gRNA. These results are based on ddPCR analysis. A similar range was measured using a surveyor nuclease assay. Gene editing by surveyor assay and ddPCR correlated with the frequency of PD-1–negative cell phenotype by flow cytometry (i.e., 25–55 percent). Similarly, increased editing frequency at the *TRAC* and *TRBC* loci was noted in both donors by the surveyor assay and ddPCR when the amount of gRNA was increased. The disruption levels were similar between the two donors. This analysis is being expanded to detect the frequencies of gene/allele disruption in a larger pool of healthy donors. The investigators do not expect 100 percent gene editing. In the initial study with CCR5 editing using ZFNs, for example, the editing efficiency ranged from 11 percent to 28 percent. They note further that substantial variation is anticipated when multiple donors are examined on experiments conducted over various dates.

Animal studies that assessed safety of triple-editing similar to the proposed Phase I trial using NY-ESO-1 used the immunodeficient NOD/scid/γcnull (NSG) mouse, which is an excellent xeno-transplantation model to measure the *in vivo* repopulation of human T cells. Following engraftment, the human cells can be maintained in NSG mice for at least two months or until fatal xenogeneic graft-versus-host disease (xGVHD). Typically, there is an initial loss of T cells, then a subsequent expansion of a clonal population of mouse-reactive xenogeneic human T cells. This model is widely used in preclinical studies to test efficacy of human T cells that are genetically redirected toward specific targets. However, testing safety of human T cells in this model is challenging due a number of reasons, including antigen distribution, differences in biology of antigen processing and presentation between human and animal cells, limited recognition of animal antigen by the human TCR, and lack of a complete immune system in the NSG mice. The investigators plan to conduct a toxicology study testing safety, efficacy, and biodistribution of the investigational cell product prior to the IND submission to the FDA. Safety assessments include clinical observation, weight, mortality, clinical pathology, organ weight, gross pathology, and histopathology. Experiments to test the efficacy of triple-edited NY-ESO-1 TCR redirected T cells *in vivo* in the NSG mouse model have been done. Results indicate that triple editing provides a survival advantage that is statistically superior to previous NY-ESO-1 cells.

Off-target effects of triple-gene CRISPR-mediated disruption will be analyzed in the proposed trial with the same deep sequencing that was used to identify the potential targets for the gRNAs in the two donors studied to date. Using the web-based algorithm called “CRISPR Design” developed at the Massachusetts Institute of Technology, the investigators identified 148 genes as potential off-target sites over the three targeted genes. The analysis indicates high on-target gene disruption efficiency in CRISPR/Cas9-treated samples. The CRISPR-induced indels (insertion or deletion mutations) were located in a narrow window surrounding the predicted target site, specifically, within a 20-bp window near the cleavage site. On average, 95 percent of the CRISPR-induced on-target indels are small deletions. Deletion of one nucleotide in an intronic region of the *ANK1* gene on chromosome 8 is the most frequent CRISPR-induced indel, accounting for approximately 15 percent of the total mutations. The longest indel was a 53-bp deletion. The *ANK1* gene encodes for ankyrins, a family of proteins that link the integral membrane proteins to the underlying spectrin-actin cytoskeleton. Ankyrin 1, the prototype member of this family, was first discovered in erythrocytes, but it has also been found in brain and muscles. Mutations in erythrocytic ankyrin 1 have been seen in patients with hereditary spherocytosis. The modified cell product that will be used in this research does not contain erythrocytes. Additional analysis would be required to validate whether the *ANK1* gene is an actual off-target site or an artifact due to a low-frequency sequencing error.

All potential financial conflicts are reviewed by the UPenn Conflict of Interest Standing Committee (CISC) per institutional policy. The CISC will evaluate whether Dr. June can participate in this study in the proposed capacity and/or if a management plan will need to be issued and agreed to by Dr. June. The current COI language in the informed consent is not final, but it is included as a placeholder. The final

language will be defined after the UPenn CISC review. CISC/IRB requires all financial interests to be disclosed appropriately in the consent. The final language will be reviewed by the CISC and IRB and approved by the IRB based on the CISC management plan.

Dr. Stadtmauer pointed out that in general, most research participants in the trial have a poor prognosis with conventional therapies. If a patient shows some improvement on a standard treatment during the manufacture process, they could still be given the investigational product. Past experience with this patient population shows that such patients can respond to similar study therapies, and so this would remain an option for them under the proposed trial.

Regarding the question of relative efficacy of the NY-ESO-1 T cells versus the NYCE T cells, Dr. June clarified that the PD-1-deficient cells are expected to initially have a better response in the NY-ESO-1 T cells. Over time, however, the efficacy of these cells is expected to wane, with wild-type PD-1 cells subsequently stepping in. These predictions are based on mouse studies in which early proliferation and function of PD-1-deficient TCR transgenic T cells caused potent tumor rejection, but long-term proliferation and stability of the T cells diminished over time in the absence of PD-1. The primary importance of the planned trial is the safety of multiplex editing. Adoptive therapy in this case will involve three genes at the same time, and the major issue is the incidence and consequence of translocations. The competitive repopulation strategy will be monitored using several assays, including ddPCR analysis of sorted cells, to determine whether there is a relative enrichment or depletion of cells with wild type PD-1 versus PD-1 deficiency; the associated function of the different cell populations will also be assessed. These analyses will not be able to identify which cells have wild-type checkpoints, however. In a prior study, endogenous *TCR* and endogenous *TCR* knockouts were introduced in the setting of wild-type PD-1 cells; better outcomes were seen with the *TCR* knockout (e.g., in terms of lysis and cytokine function). The investigators anticipate that a similar response will be seen with CRISPR-edited cells because competition with endogenous *TCR* will not be present.

The proposed trial will be a hypothesis-generating study; the primary endpoints will be the safety of multiplex editing and feasibility of achieving the target dose. Because all data thus far from clinical studies have been in healthy volunteers, these outcomes are unknown in cancer patients. The manufacturing process in this patient populations may also prove more challenging, especially for those who have had multiple lines of therapy.

Assays to detect genomic editing by CRISPR are done on triple-edited cells. Different approaches are used for analysis of the manufacturing product and the product after it is infused into a research participant. Analysis on a bulk basis can be done using flow-based assays. Because manufacturing is done without a single-cell assay, however, the investigators cannot say explicitly how many cells have had a triple hit. Analyses on single-edited cells have not been done but would be used to provide a definitive answer as to how many gene edits the T cells have received. In a research participant, toxicity often results from clonal outgrowth of T cells, which can be assessed through cell sorting and assays to screen for genomic editing and to determine whether the event is due to an on- (or off-) target effect.

Dr. Melenhorst explained that research participants will be followed closely for T cell expansion using a real-time PCR assay for the TCR and a ddPCR assay for the gene knockout. Deep sequencing will be done on any clonal expansion of these cells to identify any off-target effects.

Dr. June noted that the details of his COI cannot be provided at this point but will be available later in the review process. Once the RAC approves the protocol, UPenn can approve a final management plan for disclosure and mitigation of the COI. The management plan is derived by the UPenn CISC upon submission of the finalized protocol and consent documents. Dr. June explained that as scientific advisor, he has not role in patient care, consent, or selection. These activities are done by physicians who have no conflicts of interest. Dr. June will be involved in reviewing but not approving data submitted to the FDA, and he will be able to be a co-author but not the primary author on scientific manuscripts on which he works. Dr. June is not the FDA sponsor on the proposed trial, as he is for other protocols where there is no conflict. In this case, UPenn will hold the IND. An extensive management plan will be prepared for this

study, and it will include a detailed description as to what the scientific advisor can and cannot do in this role.

Dr. June was not aware of any other COIs for the other investigators or study sites but noted that if there are any, they will be fully disclosed. From the perspective of the study team and participating institutions, the plan to manage COIs is acceptable and appropriate. The protocol chair, Dr. Stadtmauer, has no conflicts. The engineered T cells will be manufactured for all study sites in accordance with GMP at the UPenn Cell and Vaccine Production Facility. The UPenn CVPF is accredited by the Foundation for the Accreditation of Cellular Therapy and has extensive experience and worldwide experience in the manufacture of such products. UPenn is the only institution among the study sites with the qualifications to manufacture the cells. Dr. June acknowledged that UPenn is conflicted. This COI is addressed and mitigated, however, per the management plan and strategy.

Dr. June clarified that the University of Pennsylvania alliance with Novartis is for CAR T cells. The proposed trial will not be funded or sponsored by Novartis. UPenn has no direct relationship with Life Technologies, which holds technologies licensed at this point to Adaptimmune and to Novartis for the growth of T cells. These technologies will not be used in the CRISPR study, and no funding will be provided by Life Technologies. The Parker Foundation is a not-for-profit organization. Provisions for commercialization of the investigational product through this organization are being developed and negotiated. The language in the consent document will be revised to clarify these relationships and the roles of each in association with this study.

Dr. June noted that none of the costs associated with the investigational tests, procedures, and products are charged to the research participant or insurance company. Costs of standard-of-care testing and exams are charged to the research participant's insurance provide. Research participants are not responsible for those costs, and if the research participant does not have insurance, UPenn will cover those expenses. Different housing options are available to study participants and families. Hope Lodge is located close to the UPenn campus and provides housing accommodations to research participants and their families; this facility is not affiliated with the university. In addition, many local hotels offer discounted prices, and a list of families in the area that house patients for free will be provided to participants. The consent will be revised to clarify these points.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

<u>Clinical and Trial Design</u>

- Consider collaborating on more sensitive assays to detect translocations that could possibly result from the simultaneous editing of three different loci by the CRISPR/Cas9 system.
- Given the existence of financial conflicts of interest (FCOI) at the University of Pennsylvania, consider additional mechanisms that could be used to mitigate FCOIs in the trial on the part of the University of Pennsylvania and its investigators. Such mechanisms could include, for example, blinded data analysis and/or use of an independent data safety monitoring committee (that is independent of individual and institutional conflicts of interests) to independently evaluate whether potential participants meet inclusion and exclusion criteria.
- Consider reviewing the policies for reimbursement of travel and housing costs of research participants and their families. Consider including payment for cancer tests needed for this study.

<u>Ethical, Legal, and Social Issues</u>

- Consider adding a statement in the informed consent document indicating that although a research participant may withdraw from or be taken off the study, it may be impossible to reverse the effects of the infusion or remove the cells.

- Strongly urge adding details of the FCOI of all parties in the informed consent document.

- Clarify the role of Novartis (or lack thereof) in the informed consent document.

- Consider adding language in the informed consent document:
    - Explaining that this protocol involves the first-in-human use of the CRISPR/Cas9 system.
    - Changing the term "study doctor" to "investigator."
    - Clarifying that this is a Phase I trial, and it is very unlikely that the research participant will receive any benefit.

G. Committee Motion 3

Dr. Whitley summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Whitley requested a vote, and the RAC approved these summarized recommendations by a vote of 13 in favor, 0 opposed, 0 abstentions, and 0 recusals.

V. Updates in Manufacturing CTL019

Presenter: Carl June, M.D., University of Pennsylvania

Participant: J. Joseph Melenhorst, Ph.D., University of Pennsylvania

A. Presentation by Dr. June

Dr. June provided an update of the results of clinical trials of an investigational personalized cellular therapy, CD19 CAR T cells, and the status of the manufacture of such T-cell products for domestic and international studies. For academic research purposes, the manufacture of CD19 CAR T cells (CART19) is done at the University of Pennsylvania's CVPF. Novartis has undertaken commercial-scale manufacturing of CTL019 (under the name of tisagenlecleucel-T). The decision to present this update was triggered by reports of several adverse events involving CD19 CARs manufactured by UPenn and Novartis. The presentation focused on challenges in early-stage therapy with CD19 CARs and how these challenges can inform other trials that are underway, including how product purity may affect patient safety and outcome. This work, including the analysis of patient samples and manufacturing samples, has been done by the UPenn team in close partnership with Novartis.

Clinical trials of second-generation CART19 as a possible treatment for CLL began in 2010, with results reported in the *New England Journal of Medicine* the following year. Subsequent studies in children and adults with ALL were conducted in 2012 and 2013. In 2014, results of the first 30 treated ALL patients, five of whom were adults and 25 of whom were children, were published; the presentation to the RAC included details about a long-term complication in one of these patients, who was in a complete remission at the time these results were reported. In 2015, another case report of a patient treated with CTL019 for a CD19-negative myeloma was published and showed a variant result. Two mechanisms of resistance to CART19 experimental therapy leading to relapse have been identified thus far: One is the evolution of initially CD19 positive leukemias into CD19-negative variants; and the other being the transduction of leukemic B cells with the CD19 CAR.

CTL019 manufacturing begins with a patient's own T cells, which are collected via apheresis; the apheresed cells are then reprogrammed with a gene transfer technique that is designed to instruct the modified T cells to target and kill tumor cells. The engineered cells contain a CAR that is designed to bind to the CD19 protein on the surface of B cells, including the cancerous B cells that characterize several

types of leukemia. A signaling domain built into the CAR promotes rapid multiplication of the modified cells that can grow to more than 10,000 new cells for each single engineered cell patients receive. The lentiviral-transduced cells are expanded in a process that takes 5 to 10 days and then harvested. The final cell product is cryopreserved; prior to infusion, release tests are done, and the modified cells are re-infused into patients with or without conditioning chemotherapy, depending on the design of each individual protocol.

The issue of pharmacovigilance, specifically, the safety of this approach, has been studied for nearly two decades and began in 1997 with the Cell Genesys trial of first-generation CAR T cells that were modified using a retroviral CD4z (zeta) CAR. Other trials involved T cells engineered with lentiviral antisense HIV *env* and zinc finger nucleases. Results of the 10-year follow-up of patients in the early CAR T cell trials were published in 2012. The patients from these trials remain engrafted with the CARs at levels of 1–3 percent in the peripheral blood and have had no complications from this intervention. To date, more than 300 patients have been treated with CAR T cells in 11 trials, including 219 patients treated with CART19/CTL019. More than 1400 patient-years of safety data have been collected through a long-term destination protocol, in accordance with FDA policy requiring a 15-year follow-up for such gene transfer trials. Results show no incidence of gene toxicity across studies, cell products, and patient groups. Dr. June commented that these findings are indicative of a better safety record than chemotherapy, where one would expect to see, for example, secondary AML with the same type of follow-up.

CAR trials are now being conducted around the world. A search of the clinical trials.gov website for the term “chimeric antigen receptor” indicated 130 ongoing CAR trials as of June 2016, in stark contrast with only three trials of second-generation CARs then underway in 2010, thus reflecting a marked growth in this technology. Most current trials are in North America and Asia, with a small number in Europe, Australia, and India. Dr. June noted that the initial UPenn study in relapsed and refractory ALL pediatric patients has been globalized by Novartis and is called the ELIANA trial.

Although a number of companies and academic centers are now conducting clinical trials with engineered cells, there currently are no established industry standards on how cells are isolated, processed, and cryopreserved; how cells are counted; and how dosing should be done (e.g., number of cells per kg, per body surface area or flat dosing). Guidance is emerging, however, for the commercial production, testing, and international use of these kinds of therapies, which are complex and evolving. For example, FDA guidance for commercial-scale T cell manufacturing was released in April 2016. Dr. June noted that Novartis and UPenn follow a rigorous set of protocols to demonstrate comparability following the technical transfer of methods for CAR T cell manufacture and analytics from the academic to the commercial setting. Initial data from Novartis indicate that consistency in multiple processes, including transfer of the product from the manufacturing plant to study sites for administration to patients, is possible for international trials. Similarly, UPenn has a number of ongoing multi-site trials at several academic institutions that have successfully followed these protocols.

Overall results for patients treated with CART19 are encouraging but also suggest mechanisms of resistance underlying relapses following treatment. More than 90 percent of the first 60 children with refractory or relapsed ALL had a complete remission (CR) at day 28 post-dosing. Twenty-three of these patients subsequently relapsed, and the types of relapses can be divided into two groups, early and latent relapses. Early relapses are associated with loss of B cell aplasia (BCA) and CD19+ leukemias; this has occurred in nine research participants. Latent relapses are associated with target loss (i.e., CD19-negative leukemias) and maintenance of BCA; this has been reported in 14 research participants. All but two relapses occurred within a year after dosing. Two of the 23 relapses occurred after bone marrow transplantation (BMT) in subjects who had BMT during remission. There has been only one case to date of CTL019-treated leukemia where the CAR T cells are known to be responsible for the relapse. The mechanisms for these relapses include CD19 escape, which involves the evolution of CD19-positive leukemias to C19-negative leukemias in one instance, and the other being the transduction of leukemic B cells with the vector expressing the CAR (termed CAR B cells). Given the role of BCA in both early and late relapses, BCA appears to be a very good surrogate for *in vivo* activity of the CARs. Early relapse is due to a failure of the CARs to maintain BCA, while patients with late relapses maintain B cell aplasia,

indicating that the CARs continue to work. Normal B cells however are absent in patients with functioning CARs; a subset of these subjects, in turn, appears to generate an immune response against the CAR.

Researchers at Children's Hospital of Philadelphia (CHOP) have investigated the mechanism of CD19 escape, which involves a combination of mutations and altered splicing that favor retaining most of the CD19 protein, but loss of the CD19 binding target that is recognized by the CAR construct. *CD19* is an immunoglobulin superfamily gene with 14 exons; the CD19 antigen is expressed on most B-cell acute lymphoblastic leukemias (B-ALLs) and can be targeted by CART19. Relapses with epitope loss occur in at least 20 percent of pediatric responders. Hemizygous deletions of *CD19* and mutations in exon 2 of *CD19* have been noted among relapsed subjects; in addition, a number of the relapsed samples had alternatively spliced *CD19* mRNA, including one lacking exon 2 where the CART19 binds to its target. Transcripts of *CD19* in relapsed patients have a direct splice from exon 1 to exon 3 (thus skipping exon 2) so that the CAR can no longer recognize its target epitope. A key splicing factor (SRSF3) involved in exon 2 retention was expressed at lower levels in relapsed B-ALL subjects. Exon 2 skipping bypasses exon 2 mutations in B-ALL cells and allows expression of the N-terminally truncated CD19 variant, which fails to trigger killing by CART19 but partially rescues defects associated with total CD19 loss. These findings indicate an elegant escape of the target from the leukemic cells in the context of a C19-directed therapy. Dr. June noted the similarity with monotherapy treatment of HIV in which cells potentially retain fitness but subjects relapse due to an editing event involving the target antigen. He pointed out, however, that most relapses retain B-cell expression of CD22 and CD20, suggesting that combinatorial CAR approaches targeting other surface antigens (e.g., a cocktail of CD19 plus CD22 and/or CD20 CAR T cells) could either rescue subjects from ALL relapse or, in the future, altogether prevent relapses that occur via CD19 escape. It currently is not possible to predict which research participants will be prone to CD19 loss, but further investigation of possible predictive markers is clearly warranted.

Dr. June then focused on a recent case of a documented relapse after transduction of ALL cells with CART19. (This case has been reported to NIH, and a final report is forthcoming.) The subject is a 21-year-old man with a long history of ALL. He was initially diagnosed with pre-B ALL at age 3, for which he underwent chemotherapy between 1995 and 1999. His first relapse was in 2005, when he was given intensive chemotherapy. A second relapse occurred in 2010, when he received chemotherapy and then total body irradiation, followed by chemotherapy, and cord blood transplant. The subject relapsed again in 2012, at which point he was once again administered chemotherapy and then was infused with CTL019 in 2013 under the CHOP trial. The subject experienced complete remission and was diagnosed as being minimal residual disease (MRD) negative but he relapsed a fourth time, 9 months later, with CD19-negative ALL. Further investigation showed this individual to be a clear outlier compared with other subjects, as reported to the FDA and NIH in March 2015.

The pharmacokinetics of CART19 in the first 30 patients with ALL showed a rapid increase in the CARs, which appears to be driven by CD19, followed by decay (decrease) in the CD19 CAR cell population (Grupp et al., *NEJM* 2013; Maude et al., *NEJM* 2014). PCR was run to determine the copies of CART19 per microgram of genomic DNA in peripheral blood. The subject in question had the same initial increase and peak levels of CAR T cells as others in the trial. Dr. June noted that most patients at this point are CD19 negative, which is thought to explain in part the subsequent contraction of the CAR T cells because the patients no longer have C19-positive B-cells, either normal or malignant. The subject's remission was confirmed at 3 and 6 months post-infusion and showed the typical contraction of CARs to 0.1 percent, the limit of assay detection. However, the decay in so-called "memory CARs" that occurred over a period of about 30 days in the other subjects did not happen with this individual. Instead, CAR sequences, detected in this subject through 22 months post-infusion, exhibited a bi-modal expansion localized to CD3-negative, CD10-positive cells implying that that CAR sequences were expressed, not in the T cells but on CD19-negative relapsed ALL blast cells. These findings were confirmed both in peripheral blood and bone marrow samples.

At least two potential mechanisms could explain this outcome. One is that the circulating CARs were activated and replication competent lentivirus (RCL) infected *in vivo* a minor population of ALL cells, which then expanded out. Assays for RCL infection were negative for this subject, however, thereby ruling out this as a likely mechanism. This led the investigators to the more likely conclusion that the

event occurred *in vitro* during cell culture, where both residual ALL and selected T cells in the apheresis product were transduced with the CAR construct, ultimately resulting in the post-infusion relapse.

Sequencing of the immunoglobulin H (IgH) gene, which codes for all B cell receptors, was done on the apheresis product used to manufacture the CAR administered to this subject as well as on his bone marrow at the time of relapse. Results indicated rearrangements in both the apheresis and relapse bone marrow samples. One rearrangement was several orders of magnitude higher than the others and was determined to be the subject's leukemic clone. It therefore turned out that two IgH genes were rearranged, one non-productively and one productively, which Dr. June noted is not an unusual event. In this case, the relapsed leukemia is the same as that derived from the parental (original) leukemia and thus is a subclonal variant. The investigators were not able to definitively determine the presence of separate rearrangements in more than one cell type; thus making it more likely that a single clonal cell had two rearranged IgH genes based on the results generated to date.

Further analysis identified the variant as IgHV02-70. Deep sequencing was done on the infusion product (control) and on the month 9 and month 20 post-infusion samples (peripheral blood, relapse bone marrow). This analysis confirmed the polyclonal integration site repertoire of CAR19:BB:z in the infusion product. More than >15,000 unique integration sites were detected in T cells, indicating an active apheresis product, but only 7 integration events were shared among all three specimens. None of the lentiviral integration sites in the relapsed leukemia were near what Dr. June referred to as “worrisome genes” (e.g., *LMO2, IKZF1, CCND2, HMGA2, MECOM/EVI1*). The CARB ALL cells have two integrated copies of CAR19, one on chromosome 10, next to the neuropilin gene (*NRP1*), and one on chromosome 13 in an intron in the *propionyl-coenzyme A carboxylase-A (PCCA)* gene. Although the CAR integration sites have been identified, it is not known whether these integration sites have any role in cell activation and proliferation or whether these sites simply serve as markers.

Retrospective analysis of samples from 18 ALL patients who received the UPenn CART19 product showed different tumor clonal types in two patients, including the late-relapse case discussed at length during the RAC meeting. The other patient, who had an early relapse at 2 months post-infusion, had a low percentage of CARs at the time of relapse; about 6 percent of the CAR19+ cells were leukemic B cells, but only a small subfraction of the leukemic cells (0.075 percent) were CAR B cells (in contrast with the late-relapse patient with nearly all CARB ALL cells). Dr. June noted that the characteristics of the early-relapse patient’s cells at the time of relapse resembled a very immature pre-B cell ALL phenotype.

The apheresis product of the late-relapse patient had a very high input tumor burden; nearly 80 percent of the initial leukemia cells were CD19-positive. The final infusion product was highly purified, however, with only 36 out of 4 million ALL cells identified as CD10- and CD22-positive, indicating a very low level of contamination within the standard manufacturing process. More than 99.5 percent of the infused cells were transduced T cells. It is not known whether the very small number of cells that escaped were CD19-positive or negative. The input tumor burden of the apheresis product and the purity of the final infusate of the other ALL patients also were determined. CART19 final product purity was assessed using flow cytometry; IgH sequencing was done on both bulk and CAR-sorted product. The input tumor burden varied considerably among patients, ranging from very high to relatively low numbers of ALL cells. The final manufactured products were very pure for all subjects dosed (at least 93 percent T cells). Leukemic clonotypes were in identified in the CAR-sorted product in 7 of the 18 patients using IgH sequencing; the standard flow cytometry assay was not sensitive enough to detect leukemic cells in these samples. The residual ALL cell levels in these seven patients were very low (range, 0.07–4.4 percent; median, 0.2 percent), and only two samples contained CARB ALL cells. Dr. June pointed out that the CD19-negative subclone responsible for the relapse and death of the late-relapse patient approximately 2 years after treatment was derived from a single cell. As the only case to date among more than 200 individuals experimentally treated at UPenn/CHOP, this was therefore a rare event. Given that the infused product for the late-onset patient was more than 99 percent T cells, the investigators are exploring whether cell sorting should be used during manufacturing.

Another question being explored is whether there are functional effects of CAR19 expression in ALL cells. This was tested by looking at the effect of CD19 or mesothelin (control) stimulation on the survival of

CARB ALL relapse cells. Use of CD19 to stimulate a cell that has CAR19 appears to enhance its proliferation over the short term, when compared to stimulation of the parent cell. Results also indicate slightly less apoptosis when the cells are transduced intentionally with a lentiviral vector encoding the CAR. Whether this also happens *in vivo* is not known.

Dr. June noted the following outstanding issues regarding CD19-negative relapse in cells that express CAR19:

- Was the parental cell that was transduced with the CAR CD19 positive? Some data indicate that intentional transduction of ALL cells renders them CD19 negative.
- If the parental ALL was CD19-positive, did the inadvertent transduction by CTL019 lentivirus *in vitro* cause CD19 downregulation or was the cell always CD19-negative?
- Was a CD19-negative ALL cell transduced with the CAR?
- Were CAR19 integrations in or near *NRP1* and *PCCA* sites acting as drivers or passengers?
- Did the insertion events *(NRP1, PCCA)* change the aggressiveness of the ALL?

Lessons learned thus far regarding CARB cells and tumor contamination in the context of a late relapse:

- A CD19-negative subclone derived from a single cell was responsible for the relapse of the research participant.
- In the last 30 years of experience with autologous hematopoietic stem cell transplantation, tumor contamination of the infused marrow has not been shown to have an adverse impact (with one possible exception), rather, it is residual tumor in the transplant recipient that has been deterministic of outcome.
- Because CTL019 is able to efficiently eradicate tumor *in vivo*, infused tumor burden now has assumed importance.
- Low level contamination of CART19 infusion products with ALL tumor cells is not uncommon.
- Two mechanisms of resistance to CTL019 therapy have been identified (CD19-negative B-ALL cells, CARB cells).
- At this point, relapse accompanied by CARB is a rare event, as this was identified in only two cases in more than 115 ALL patients treated to date at UPenn/CHOP.
- Given the high purity of the infused product, cell sorting is unlikely to improve outcome during manufacturing.
- Elimination of CD19-negative B cell blasts, as seen in the late-relapse patient, may require dual targeting of both CD19 and other targets such as CD22.

B. RAC Discussion

Dr. Curry asked how variable the time course for CD19 escape is. Dr. June noted that results to date suggest that CD19 escape occurs between 3 months and 1 year. In many patients, escape is accompanied by loss of B cell aplasia. A trial at Stanford is assessing the safety of CD22 CARs; once safety is established, the research may progress to investigate whether it is possible to treat patients preemptively with both CD19 and CD22 CARs to potentially reverse ALL relapse or altogether prevent escape.

C. Public Comment

Dr. Lee asked whether, for CARB, the CD19-negative assay was done with an antibody that binds to a non-overlapping epitope to CD19, and if the investigators know whether the CAR actually covers the epitope. He also inquired as to whether CD19 has been sequenced to identify its mutation. Dr. Melenhorst replied that sequencing of the patient's relapse samples found no mutations in CD19 or any other protein associated with the CD19 complex (e.g., CD81). He explained that because most commercially available antibodies recognize the same CD19 exon 2 epitope, these agents are not able to detect the CD19 protein. Western blotting needs to be done to look at the residual CD19 protein. The investigators used an antibody that recognizes an intracellular epitope on CD19, which shows lower levels of expression of the protein in the relapse samples compared with normal B cells and other leukemic cells at baseline.

In response to another question regarding the ability to detect the CAR CD19 epitope in the presence of native CD19 where interference could occur resulting in epitope masking, Dr. Lee wondered whether the presence of two copies of the CAR, and thus higher expression of CD19, in the late relapse case favored epitope detection. Dr. June noted that in other CD19 negative relapse cases, no integrated CARs have been observed except in one relapsed subject where approximately 0.3 percent of non T cells had detectable CAR sequences. The presence of the CAR sequence did not appear to provide a competitive treatment advantage because over time, the CARs decreased and the ALL cells increased.

VI. Acknowledgment of Service of Departing RAC Members

Dr. Tucker thanked the five departing RAC members for their exceptional service in advising the NIH on scientific, safety, and clinical applications of recombinant and synthetic nucleic acid research. Their 4- to 5-year tenures included participation in and leadership on several RAC panels and working groups in addition to the standing RAC. The members' contributions have informed the field of gene transfer research and have been important to both the scientific community and the public at large. Dr. Tucker presented the following departing RAC members with certificates of appreciation for their service on the RAC: Dr. Pilewski, Dr. Wooley, and Dr. Zoloth. Two additional departing members, Dr. Cannon and Dr. Kiem, were not present to receive their certificates in person. Dr. Tucker thanked Dr. Kiem, who joined the RAC in 2011 and has served as Chair since 2015. Dr. Whitley will serve as the new RAC Chair with Dr. Kiem's departure.

VII. Review and Discussion of Human Gene Transfer Protocol #1604-1520: A Phase I/II, Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) Serotype 8 (AAV8)–Mediated Gene Transfer of Human Ornithine Transcarbamylase (OTC) in Adults with Late-Onset OTC Deficiency

Presenters:	Cary Harding, M.D., Oregon Health and Science University (OHSU)
Sponsor:	Dimension Therapeutics, Inc., Cambridge, MA
RAC Reviewers:	Drs. Ross, Whitley, and Wooley
Ad Hoc Expert:	Ada Hamosh, M.D., MPH, The Johns Hopkins University

A. Protocol Summary

Ornithine transcarbamylase (OTC) deficiency, the most common of the urea cycle disorders, is an X-linked disorder that results from mutations in the OTC gene, affecting the expression or activity of the OTC protein. A deficiency in OTC activity prevents the normal flux of ammonia through the urea cycle and ultimately results in hyperammonemia that can lead to neurological damage and death. OTC deficiency presents as a severe form (i.e., complete OTC deficiency) in males shortly after birth ("neonatal onset," presenting at ≤1 month of age) or later in life for both males and females ("late onset," presenting at >1 month of age) with disease that ranges from mild to severe depending on the residual activity of OTC. The current standard of care for OTC deficiency is to limit dietary protein intake and supplement the diet with a high energy source, such as glucose. If serum ammonia is not stabilized by dietary restriction alone, ammonia scavengers that promote an alternative pathway of nitrogen removal can be administered. However, ammonia scavengers cannot completely prevent individuals from hyperammonemic crises (specifically, hyperammonemic shock), especially in the setting of acute illness such as infection. Liver transplantation is a therapeutic option for OTC deficiency but is limited by donor availability and is associated with significant risk of morbidity and mortality.

Increasing OTC activity and promoting the removal of ammonia through the urea cycle should allow patients with OTC deficiency to avoid hyperammonemic crises in the context of a well-controlled diet while reducing or stopping ammonia scavenger therapy. Furthermore, depending on the level of OTC

expression and activity achieved, patients may be able to loosen their dietary restrictions and decrease frequent, chronic medication use, which should greatly improve their quality of life. OTC gene transfer is expected to be effective for the treatment of OTC deficiency. Unlike current treatment options (dietary restriction and ammonia scavengers), OTC gene transfer offers the potential to correct the underlying deficiency for a prolonged period of time.

The investigators plan to assess an investigational OTC gene therapy product, DTX301, in adults with late-onset OTC deficiency. DTX301 contains a codon-optimized OTC gene with expression driven by a liver-specific enhancer and promoter encapsidated within an adeno-associated virus serotype 8 (AAV8) vector. The virus is normally single stranded, but the proposed construct is self-complementary; this form of the virus presents the genome in a double-stranded configuration that leads to increased transduction efficiency. AAV8 has strong tropism for the liver and transduces the liver very efficiently when administered intravenously. The proposed study is a Phase I/II, open-label, multicenter, safety, and dose-finding trial to determine the safety, tolerability, and efficacy of single IV doses of DTX301 in 6 to 12 adults with late-onset OTC deficiency. The study is designed to determine the optimal biological dose (OBD) of DTX301 using the following candidate doses: 1.5×10^{12} genome copies (gc)/kg, 3.0×10^{12} gc/kg (Cohort 1/starting dose), and 1.0×10^{13} gc/kg. Dose exploration and determination of the OBD will be assisted by a model that uses safety data to recommend the next dose. Patient enrollment will be staggered to determine potential adverse effects. A 42-day window will be used between the third patient of each dose cohort and the institution of the next dosage level. The decision to proceed to the next dose cohort will be made by the sponsor after the DMC has evaluated the safety data. Volunteers will be followed for a total of 52 weeks for the main study, with an additional 5 years of follow-up.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because it is a first-in-human study involving administration of a novel AAV vector expressing human OTC to adults with late-onset OTC deficiency that are otherwise healthy. In addition, the researchers propose to administer a liver-tropic virus for a condition where viral infection itself is the most common cause of hyperammonemic shock.

Three RAC members provided written reviews of this proposed Phase I/II protocol. The ad hoc expert did not prepare a written review and presented her comments and questions during the meeting.

Many of the reviewers' questions and concerns regarding the proposed trial were raised within the context of the controversial death in 1999 of Jesse Gelsinger, an 18-year-old patient enrolled in gene therapy trial for OTC deficiency conducted at the University of Pennsylvania. Jesse Gelsinger was the first person publicly identified as having died in a clinical trial for gene therapy. He died 4 days after being injected with an adenoviral vector carrying a corrected OTC gene; he suffered a massive immune response triggered by the use of the adenoviral vector that led to multiple organ failure and brain death. Although the viral vector that will be used in the proposed trial is different from that in the 1999 trial (i.e., an adeno-associated virus vs. an adenovirus, respectively), the severe adverse effect that resulted in Jesse Gelsinger's death was thought to be unforeseen at the time; it also was thought that the adenovirus used in the previous trial was relatively safe. However, the university had failed to report that two prior patients had experienced serious side effects from the gene therapy and did not disclose in the ICD the deaths of monkeys given a similar treatment. The death of Jesse Gelsinger and in particular the circumstances surrounding his death slowed down the progress of gene therapy and had a negative impact on the entire field for a significant period of time. The reviewers pointed to the extensive media attention given to the death of Jesse Gelsinger and noted that there may be even more media attention on another gene therapy trial to treat OTC deficiency. In consideration of this history, it is prudent to apply additional scrutiny to the proposed protocol.

Dr. Ross found the protocol, answers to Appendix M, and consent forms easy to read. Her comments and questions focused on the ICD, as follows:

- The statement, "'Investigational' means that the study product has not yet been approved by any health authorities to be sold commercially," is somewhat misleading. The description in the ICD

skips a few steps that are important for participant understanding. This is particularly true given that the study participants will be individuals who are healthy but at risk for metabolic decompensation and because there are no data to indicate whether the research itself can induce a metabolic crisis. The study agent is more than "not yet approved"; it is experimental and has not been tested previously in humans, and therefore it has not been shown to be safe or effective. Only after it has been tested and proven safe and effective can any health authority consider approval and only then can it be sold. The ICD should include these points in explaining the term "investigational" in reference to the planned intervention. Dr. Ross noted that at her institution, the University of Chicago, all Phase I study drugs must be called experimental to make sure that participants and potential participants understand the experimental nature of the drugs and that such interventions are novel.

- The consent states that the study doctors do not have a financial stake in the results of the trial and that the research is sponsored and funded by a gene therapy company. The document does not say whether the institution at which the study is being done has any conflict.
- The ICD says that approximately 6 to 12 subjects will participate in the study, which will take place in multiple medical centers in countries around the world. In contrast, the appendices say that the study drug will be administered at a single site, OHSU, but that the trial may be expanded to other locations. The investigators need to clarify whether more than one site will be involved and then make this information consistent across documents.
- Use of the term "study treatment" suggests that the intervention is known to be what is understood as a "treatment." The section of the document titled, "Study Treatment," should be re-named "Gene Transfer Study" or something less suggestive of cure to ensure participant understanding, such as "Experimental Study Procedure." The consent needs to state clearly throughout (as done later in the text) that the modified gene product has never been used before in humans. To suggest that it is a treatment is wrong. The NIH booklet, *Informed Consent Guidance for Human Gene Transfer Trials,* is subject to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and may be helpful in providing language that is appropriate for the consent. It can be found at http://osp.od.nih.gov/sites/default/files/resources/IC2013.pdf.
- The statement, "It is not known whether this drug works in humans, but knowledge gained by this research study could help others in the future," is not accurate because it is not known whether this drug is safe or whether it works. Because this is a first-in-human study and a Phase I/II trial, both safety and efficacy are being tested. The statement needs to be modified to reflect the status of the drug and nature of the protocol.
- Regarding subjects' right to withdraw from the study, it is important to explain that once the gene transfer has been administered, it cannot be removed, and that the subject is only withdrawing from being followed since the therapy is complete. It is then appropriate to explain that the investigators can only know if the study agent persists by sampling and to encourage ongoing sampling, even if the participant refuses to participate in further research data collection.
- The "End of the Study" section of the ICD suggests that participants will only be invited for LTFU if this phase of the research is given regulatory and ethical approval. In contrast, the protocol states that the investigators hope to follow participants for an additional 5 years without mentioning whether this will occur only with regulatory and ethical approval. This issue needs to be clarified and aligned between protocol and consent form. Dr. Ross noted that FDA often recommends longer follow-up to look for rare late AEs, including malignancies. The consent needs to explain why long-term follow-up is necessary.
- The consent correctly states that the risks of the investigational product are unknown because this is a first-in-human trial of the product. The description of the potential risks is minimal, however. More serious side effects are possible, which is why subjects will be hospitalized and observed very carefully following infusion of the study drug. The investigators should consider adding side effects seen in similar studies and other studies using AAV vector to the consent document. For example, the protocol mentions risk of vector-induced hepatitis and death and includes safety stopping criteria, but none of these are listed in the ICD. The investigators also need to keep in mind that the death of Jesse Gelsinger will be taken into account for any studies of OTC deficiency.

- DTX301 study product will be made available for this trial at no charge, and participants will not be asked to pay for any study procedures. All subjects will be reimbursed for travel expenses and will be given a small payment for taking part in this study. The consent needs to provide specific amounts for study participation and state when payment will be given and whether prorated compensation will be offered.
- The rationale for using a home healthcare agency for research purposes is not clear and needs to be specified (e.g., to remove some of the burden of subjects' having to come to the research site every 4 days). It appears that the home healthcare agency will be used only to collect samples and that no vital signs or other participant interaction will occur. If that is the case, it should be permissible. However, if the personnel are expected to do more (e.g., help the participant fill out surveys), then they must be listed in the IRB and trained with human subjects protections and good clinical practice training. The investigators need to confirm that agency staff will only draw a blood sample and collect bodily fluids, and that no other activities will be performed.
- Only one CV, that of Dr. Harding of OHSU, is included in the materials submitted for review. CVs of other members of the study team should be provided.

Dr. Whitely had the following general comments and questions:

- Although utilization of AAV8 has been employed in other gene transfer studies with no evidence of toxicity (particularly in the correction of hemophilia), no data were presented in the materials provided to indicate dose-limiting toxicity of the current AAV8 that expresses the OTC gene. What do the investigators anticipate in this and similar studies, particularly given that the liver is the target organ for gene transfer delivery and, therefore, could be the subject of toxicity?
- The continual reassessment model (CRM)/adaptive design seems appropriate for this study. However, the actual number of patients is limited with respect to determination~~s~~ of safety. Specifically, it appears as though Cohort 1 will receive 3.0×10^{12} gc/kg. Is the dose of 1.5×10^{12} gc/kg the lower de-escalation dose that will be used should toxicity appear? Is the upper dose of 1.0×10^{13} gc/kg an adequate range to assess potential effects of the intervention? Should a wider range of AAV8 delivery be considered?
- The initial dose for administration has been targeted predicated upon murine data. To what extent has such modeling proved effective in prior AAV8 gene therapy studies? Should the lower dose be used initially?
- It appears as though the dose has been determined according to the investigators' presumption of "clinical efficacy." The investigators need to keep in mind, however, that this is a purely research study and that the participants are volunteers who should not anticipate efficacy for their participation. This needs to be stated clearly in the ICD.
- The investigators will collect saliva, blood, and urine to determine excretion of virus over the course of the 52-week study. Would it be worthwhile to attempt to obtain vaginal swabs and semen to determine risk for excretion in the genital tract?

1) If the vector induces altered liver function (i.e., as related to elevated levels of ALT), oral steroid treatment will be considered. Are there alternative etiologies to altered liver function than immune hepatitis? For example, could altered liver function be related to cytokine release syndrome (CRS) involving the liver?

- The role of the data monitoring committee (DMC) needs to be clarified, including whether the DMC will be responsible for making judgments as to moving to the next dosage level or whether this decision ultimately will be made by investigators at the sponsor (Dimension Therapeutics).
- In the "Treatment Period" section of the graphic in the protocol describing the study design, reference is made to dose Cohorts 1 to 4. Other information in the protocol implies that there will be only two dose cohorts. The number of planned cohorts therefore needs to be clarified and made consistent across documents.
- Regarding the OBD, some sections of the protocol state that the maximum tolerated dose (MTD) will be determined when a dose cohort experiences a DLT greater than or equal to 25 percent. When only 2 to 3 patients are enrolled per cohort, a DLT involving 25 percent of subjects can't be achieved. It would have to be either 50 percent or 33 percent. The investigators need to clarify if

they plan to continue to accrue patients into a cohort for a dose that is safe. If that is the case, it should be elaborated upon in the protocol.

- The following issues need to be addressed in the ICD:
    - Under "Benefits of Treatment," there is no indication that administration of DTX301 will be beneficial to patients. Offering them that hope is inappropriate, and the language therefore needs to be revised to state otherwise.
    - Exposure of family members to those who receive DTX301 may be greater than anticipated, albeit AAV8 does not replicate.
    - It is not clear who will adjudicate payment for complications should the PI and the patient disagree on causality. This person or entity needs to be specified.

Dr. Wooley noted that studies of DTX301 have been conducted in mice, specifically, a mouse model for OTC deficiency, the *spf^{ash}* (sparse fur) mouse. Preliminary results from these studies and clinical experience with other AAV8 products were used to assess potential risks and determine the starting dose for the proposed trial. It is not clear, however, whether there have been any studies of DTX301 in nonhuman primates (NHPs). The monkey deaths in the studies conducted prior to the 1999 trial predicted the potential serious adverse effect for Jesse Gelsinger, but these results were not made known to the participants.

Per the protocol, the vector could persist for years or even a decade, but this would not give a life-long cure. Dr. Wooley asked whether the vector could be re-administered to a person later in life and whether any plans are in place to re-administer other serotypes to avoid a neutralizing immune response.

Dr. Wooley also asked whether the study participants will maintain their normal diets and medications during the trial. She had the following comments regarding the ICD:

- The description of the DTX301 vector in the "Study Treatment" section confuses AAV with adenovirus and contains inaccuracies. A revised description was provided (with suggested changes in italics): "The DTX301 vector is *made from* a virus called *Adeno*-associated virus (AAV). AAV is a common virus *found throughout the body in natural infections and is not currently known to cause disease*. The AAV vector used in DTX301 to deliver the OTC gene has been *modified* in the laboratory so that it cannot reproduce *itself* once it is in your body."
- The "Study Withdrawal" section needs to inform subjects that the virus will remain in their bodies even if they leave the trial.
- The death of Jesse Gelsinger should be mentioned with an explanation of how this study is different from the previous one (e.g., use of a different vector, AAV vs. Adenovirus).

Dr. Wooley noted that it was difficult to evaluate the following additional issues and aspects of the proposed trial because the information was contained in a confidential file that could not be accessed for review:

- Details of the plasmid construct and manufacturing process and controls
- Ongoing nonclinical studies
- The mouse model for efficacy
- The study objectives and clinical endpoints
- Safety and efficacy assessments
- In clinical trials with AAV gene transfer, a transient rise in liver transaminases and concurrent decline in transgene expression has been observed. This has been hypothesized to be due to the activation of capsid-specific cytotoxic T lymphocytes and destruction of transduced liver cells. Discussion of the precautions for this risk was confidential.
- Measures to control or reverse effects
- Contraception instructions
- Number of subjects to be enrolled
- Specific exclusion and inclusion criteria

C. RAC Discussion

The ad hoc expert, Dr. Hamosh, found the study design to be appropriate to answer the research questions and agreed in principle that AAV8 is the correct vector to use for the planned gene transfer product and study. She had the additional following comments and questions:

- Dr. Hamosh was concerned that the cutoff for the exclusion criterion for ammonia, which is equal to greater than 100 µmol/L, is too high for the proposed trial. She noted that many patients with OTC deficiency have ammonia levels between 10–30 µmol/L, which is closer to the normal range. She pointed out that one of the confounding factors in Jesse Gelsinger's case was his elevated ammonia level on the dosing day, and that, as a clinician, she would not give patients with a 100 µmol/L (or greater) ammonia level an experimental therapy. Other issues to take into consideration are that hospitals have variable normal ranges for ammonia and that each patient has their own the "normal" or typical range. The investigators should consider a lower cutoff for inclusion (e.g., up to two times the normal level) in the proposed trial or, as an alternative, use the individual subject's normal outpatient ammonia level as a guide for determining whether the subject is eligible to participate. Dr. Cho suggested developing an inclusion criterion that uses the ratio between the patient's normal ammonia level and the screening day level. A mechanism should be written into the protocol to take into account variability in ammonia levels in patients as an added safeguard, instead of having a hard cutoff of 100 µmol/L.
- Several of Dr. Hamosh's adult patients with late-onset OTC deficiency have presented with hyperammonemia or hyperammonemic crisis triggered by the combination of an infection and a steroid. Use of oral steroid treatment for possible vector-induced immune hepatitis could increase catabolic stress and place patients at further risk of a crisis or coma. Dr. Hamosh advised strengthening or providing more specific guidance regarding hospitalization, including for steroid use, as an added safeguard for close monitoring. Because some patients with late-onset are very stable and rarely get sick, they often do not know their own triggers and may not realize when they become ill in relation to their participation in the study.
- The investigators were asked to clarify if the ultimate goal is to use this vector product for complete OTC deficiency and if not, if this is because of concerns about possible cross-reactions.
- Additional information was requested as to how pill burden has been addressed as an issue of compliance and discomfort.
- Given the importance of limiting protein intake in this patient population, Dr. Hamosh asked about the protein burden (capsid protein) of each of the three doses of DTX301, which will be administered as a single infusion.

The following additional questions, concerns, or issues were raised by RAC members during the meeting:

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed. They went through their comments and the investigators' responses to their queries and suggestions.
- To the investigators' knowledge, none of the study physicians, institutions, or sponsor has any financial conflicts of interest for this research. If this were to change, full disclosure would be needed in the protocol and consent, and additional safeguards to mitigate any COI would need to be in place.
- Dr. Ross stressed the importance of clearly identifying the potential risks of participation, including death related to the infused product. Death is one of the criteria for suspending enrollment, but it is actually a risk of participation in this research study.
- Per the written comments, the investigators need to be careful to not refer to the study and the intervention as a "treatment." This is a gene transfer protocol to assess an investigational/ experimental intervention.
- Dr. Whitley asked if there are sufficient data about the use of AAV8 in the hemophilia studies to be able to predict which toxicities will likely be encountered in the proposed trial and to properly educate the data monitoring committee about the potential risks of the vector for this patient population.
- Dr. Whitley asked the investigators whether the dose range, which seems to be relatively narrow, could impact accrual and whether there are any plans to push the dose higher (or lower). He supported use of an adaptive design, as proposed, given the limited number of patients expected to be available for this research.

- The protocol will collect blood, urine, and saliva to assess excretion of AAV. Dr. Whitley noted that is not clear if AAV can be transmitted sexually and revisited the question about whether semen and vaginal swabs should also be collected as a precautionary safety measure. He cited the recent finding that the Zika virus can be sexually transmitted as a reference.
- The protocol includes extensive monitoring and clinical evaluations, including blood sampling, at multiple time points over 52 weeks. Dr. Cho asked if any of the monitoring visits or procedures can be done locally, or if everything needs to be done at the study site. If the local treating physician is involved, how well would this person be educated about the trial and what is entailed for monitoring?
- Dr. Whitley asked the investigators to make sure the DLT criteria for number of affected subjects is changed from 25 percent given that each cohort is expected to enroll only three subjects.
- The RAC remained concerned about the lack of information in the consent document about the death of 18-year-old Jesse Gelsinger in the prior gene therapy trial for OTC deficiency at UPenn. Dr. Wooley reviewed the details of the case and how his tragic event, which many thought could have been prevented, set back the field of gene therapy and compromised the public trust. There have been no other registered trials for this disease, and the proposed study will be the first gene transfer trial for OTC deficiency since Jesse's death. One of the controversial aspects regarding Jesse's case and the trial in which he was enrolled was that monkeys died in preclinical studies of the vector product, foretelling that death could be an outcome for patients in the clinical protocol; however, the monkey deaths were not reported or disclosed at the time, further exacerbating the case. Dr. Zoloth and other RAC members endorsed the reviewers' recommendation to include the story of Jesse Gelsinger in the ICD to fully inform potential (and subsequently enrolled) subjects of this history and to recognize Jesse's life, the risks he took, and the lessons learned from his case.
- The DTX301 vector is made from adeno-associated virus, in contrast with the investigational product in the UPenn trial, which was made from adenovirus. AAVs have had a good safety profile in clinical trials. DTX301 has been studied in the mouse model for OTC deficiency but not in nonhuman primates. Dr. Wooley inquired as to how closely the spf^{ash} mouse model mimics the disease in humans and what happens to these animals' ammonia levels when they are administered DTX301. Studies of DTX301 in NHPs could inform the planned research. Dr. Zoloth underscored the importance of such a study by pointing out that the signal (death) for the prior vector was seen only in NHPs, not in mice. Dr. Wooley suggested testing the starting dose for the proposed trial (3.0×10^{12} gc/kg) or perhaps first assessing the next lower dose (1.5×10^{12} gc/kg).
- Dr. Wooley followed up on Dr. Hamosh's comments regarding the potential for a viral infection to trigger hyperammonemia and hyperammonemic crises in patients with OTC deficiency. AAV8 has strong tropism for the liver, the site of ureagenesis. The starting dose for the proposed trial is somewhat higher than that used in preclinical studies of AAV8 and prior clinical studies in hemophilia patients. Patients with OTC deficiency may have worse outcomes than hemophilia patients due to the nature of the disease. In addition, responses in animals may not inform outcomes in humans given the significant difference between mouse and human livers in terms of toxicity. Additional information was requested regarding the risks of AAV8 in animals and humans and the highest dose of AAV8 ever used in a human.
- Dr. Pilewski asked if the relationship between the percentage of cells in the liver that have to express OTC to get biochemical correction is known. Alternatively, if the transgene is expressed abundantly in a small percentage of hepatocytes, is that sufficient to correct the problem, or is widespread expression needed even with a low copy number? Dr. McCarty inquired whether transduced cells that produce OTC have a survival advantage over non-transduced cells in the liver.
- Dr. Cho noted that the sparse fur mouse model appears to complement the genetic defect in humans and to be very good for assessing efficacy. However, this animal is not a good model for the hyperammonemic crises and other toxicities in humans with OTC deficiency, whether these symptoms are due to the underlying disease or the experimental vector. This lack of similarity raises concerns about use of the model to assess safety of DTX301.
- Dr. Cho was concerned about the dual goals of the proposed research, to assess the safety of DTX301 and to determine the optimal biological dose by giving subjects a potentially

pharmacologically active dose based on preclinical data. She advised addressing safety first and then moving to the efficacy issues.

- The Committee acknowledged the differences between the prior and the new investigational products, in particular the vectors used, but pointed to the failure to fully disclose the potential risks to participants in the earlier study. Dr. Zoloth commented on another error in the design of the UPenn trial, which was to test the investigational product in a relatively healthy cohort of patients with late-onset OTC deficiency instead of in neonates, who have a severe form of the disease and are at much greater risk of serious outcomes with onset of a metabolic crisis, including death. She raised similar concerns with the proposed Phase I trial, which will enroll the same cohort as the UPenn study, thereby exposing otherwise healthy persons to the risks of a novel AAV vector product without the potential for direct benefit to the individual subject. It is not clear why this first-in-human study of DTX301 is not being conducted initially in symptomatic patients who are not doing well and have failed conventional therapies and thus might have a small chance of benefit, even though this is not the primary aim of this trial. Upon further discussion, Dr. Zoloth noted that investigators described patients who have more serious or more frequent manifestations of the disease than presented in the protocol. The characteristics of the cohort need to be clarified and made consistent across documents. Dr. Hamosh followed up Dr. Zoloth's and the investigators' comments and suggested that the team consider modifying the inclusion criteria to enroll patients who have had, for example, two or more episodes of hyperammonemia. Individuals with late-onset OTC who present with only one episode may not be appropriate given the risk/benefit ratio to this research.
- Dr. Zoloth recommended that committee members read a 2009 article by James Wilson, who was an investigator on the UPenn trial and who also did preclinical work on the mouse model. She found the article, "Lessons Learned from the Gene Therapy Trial for OTC," published in *Molecular Genetics and Metabolism*, to be an important read and relevant to issues raised regarding the proposed trial, including patient selection, financial and other COIs, safety monitoring, and study oversight.
- Dr. Wooley located the minutes of the 1995 RAC meeting that included the review of the protocol in which that Jesse Gelsinger was enrolled. One of the primary reviewers of that study was against approval of the trial for several reasons: the toxicity of the investigational product, that the virus could not be repeatedly administered to achieve a long-term effect, and a target population that was mostly asymptomatic. Despite these concerns, that reviewer ended up voting in favor of approval with the Committee. Dr. Wooley noted that in viewing a subsequent interview, the reviewer appeared to clearly have regretted his vote. In another interview, Jesse's father said that he is not against gene therapy, but that researchers need to "do it right," by proceeding slowly in a step-by-step fashion to have a solid understanding of the technology and its risks in humans. The next step before launching the proposed clinical trial, which is a safety study, should be nonhuman primate studies at the planned starting dose or a dose that is a half-log below any AAV8 dose tested to date. With these additional preclinical studies, the vector product will then be tested in two different species. The dose could be increased later if warranted. Dr. Wooley commented that if this additional step is not done, and another death or tragic event happens again, the same questions and criticisms will be raised. Dr. Zoloth agreed and pointed out that the same concerns raised by the reviewer in 1995 were raised again, with the noting in the current review of the importance of the NHP study.
- Dr. Donahue commented that the lack of toxicity of the adenovirus in mice may have been a dosing, not a species, issue. The likely reason why there was no signal in mice was that the dose was 50 times lower than that given to the monkeys. The preclinical safety studies in mice were thus inadequate. This distinction should be taken into consideration since the motivation for the recommended NHP AAV8/DTX301 studies appears to be the previous adenovirus work. It may be that the doses in the recent mouse NHP AAV8/ DTX301 studies were not high enough to generate any toxicity. Dr. Whitley noted two animal species, rather than one, are typically used to define toxicity. The RAC could recommend preclinical toxicity studies in two animal species at the highest dose anticipated to be used in humans prior to moving forward to human studies.
- Dr. Zoloth recommended that the DSMB attached to the protocol include an ethicist and a patient advocate so that the committee is composed not only of scientists and clinicians to review the

data but also members to assess the ethical, social, and legal implications of the research as well.

- Dr. Cho noted that the list of symptoms considered DLTs does not include hyperammonemia. She asked how the investigators will determine the threshold for whether or when hyperammonemia would be a DLT.
- Dr. Hearing asked if a plan is in place should any patients develop liver cancer (e.g., monitoring for AAV integration in tumor tissue).
- Dr. Wooley recommended that the local IRBs be provided the confidential version of Appendix M as part of their review of the protocol to ensure they have the full complement of data and information when considering approval at their individual sites.

D. Investigator Response

1. Written Responses to RAC Reviews

Dr. Harding noted that the documents shared with all RAC members included a full unredacted copy of the protocol, the ICD, and a redacted version of Appendix M.

Prior to enrolling patients and throughout the conduct of the trial, investigators from OHSU or any other potential participating clinical study institution will be asked to disclose financial interests to the sponsor in writing on a financial disclosure form. Financial disclosure will be collected in accordance with CFR Title 21 Part 54, Financial Disclosure by Clinical Investigators. The investigators are not aware of any financial COIs with Dimension by potential participating clinical institutions.

In cases where the PI and the patient disagree on causality and related payment for research-related injury, the study sponsor, Dimension Therapeutics, will employ an independent third-party medical consultant to adjudicate the causality of complications.

At the time of the submission of Appendix M, one investigator, Dr. Harding, had been identified to initiate the RAC submission. Additional sites will be needed to ensure timely enrollment into the study. Once these sites have been selected, NIH will be updated with the appropriate information. Patients will not be enrolled at any new center prior to completion of the NIH notification per the requirements in the NIH OSP *Federal Register* notice (effective April 27, 2016) detailing the revised procedure for human gene transfer trials subject to *NIH Guidelines.*

This trial is expected to be a global study with sites in North America and the European Union. Reimbursement rates will be negotiated with each institution in accordance with the regulations for each country. The ICD will be customized for each center and will list the exact amount received for each study visit. The payment will be prorated per visit based on the time the patient must commit. The intent is for patient payment to be consistent across all study sites, but payments may need to be adjusted per local laws and regulations in addition to feedback from the IRB or IEC (or similar body). The IRB and IEC must approve the patient compensation program prior to it being implemented at the site. It is anticipated that patients will be reimbursed in a timely fashion after each study visit over the course of the clinical trial.

Dr. Harding acknowledged that a single peripheral infusion of an AAV8 vector encoding the human wild-type OTC gene cassette to a patient with stable OTC deficiency can cause safety events related to either the excipients used in the drug product, the protein AAV8 capsid, and/or the product of the gene cassette. The excipients in the formulation are commonly used and in the ranges typically used and are not expected to have any AEs. Based on clinical experience, the safety profile of DTX301 is most likely to reflect reaction to the AAV8 capsid.

One potential safety event from AAV vector administration is a transient and self-limiting elevation of liver transaminases. The elevation in liver enzymes is thought to be due to an immune reaction to the AAV capsid proteins, which may reduce expression of the transgene. In one study, asymptomatic transient elevations in serum liver enzymes, possibly as a result of a T cell–mediated immune response to the AAV8 capsid, were observed in 4 of 6 patients in the high-dose group (2×10^{12} vector genomes [vg]/kg)

but resolved over a median of 5 days after prednisolone treatment. These transient elevations of liver enzymes occurred between weeks 7 and 10 after AAV8 vector administration and are not considered to reflect serious tissue damage or to meaningfully impair the liver function. As reported in other gene therapy trials, treatment with steroids, if initiated as soon as liver enzymes begin to rise, appears to correct this immune-mediated response and preserve expression of the transgene.

Subject in the trial could mount an immune response to the OTC protein encoded by DTX301, but this is not expected. Preclinical studies show only transient chemokine and cytokine expression and leukocyte recruitment in the livers of mice, with no associated significant toxicity or inflammation. Patients with late-onset OTC deficiency typically express low levels of the OTC protein and therefore should be immunologically tolerant to the vector-expressed OTC protein and should not be expected to elicit an immune response to the OTC protein. Immune responses to therapeutic proteins expressed in a gene therapy context have not been common to date. Based on available information, toxicity is not expected from expression of the OTC transgene in liver.

Another potential toxicity could result from supranormal levels of expression of the transgene. However, based on the mechanism of action of the OTC enzyme, no exaggerated pharmacology is expected. Data from two published papers support the potential safety of supranormal expression of OTC in the mouse model of disease. One of these papers described supranormal expression of the DTX301 product in the spf^{ash} mouse model, with expression of the transgene approximately 500 percent greater than the expression levels in wild-type mice; despite this high expression level, no adverse findings in the spf^{ash} mice were reported. Another paper by the same group reported no adverse findings when expression of the OTC enzyme was approximately 150 percent of wild-type levels of OTC enzyme.

Direct assessments of the potential toxicity from DTX301 are ongoing; a formal good laboratory practice (GLP) toxicology/biodistribution study in C57Bl6 mice and a pharmacology study in spf^{ash} mice with safety endpoints are underway. These studies are investigating a DTX301 product that is similar to the GMP clinical product and are designed to understand the toxicity from both the capsid and expression of the transgene. The ongoing pharmacology study in spf^{ash} mice includes doses up to 1×10^{13} gc/kg, the highest planned clinical dose, which is projected to result in supranormal levels of OTC enzyme. Dimension's ongoing mouse toxicology study will include levels up to 7×10^{13} gc/kg (i.e., 7 times more than the highest planned clinical dose). The nonclinical program will evaluate supranormal levels of OTC enzyme in both normal animals and the model of OTC deficiency. The results of the ongoing preclinical studies will be submitted in an IND application and to the ethics committees to support the first-in-human dosing of DTX301.

Dr. Harding explained that unlike other more traditional small molecule or biologic therapeutics that require repeated dosing over time, gene transfer is a one-time therapeutic intervention due to the expected emergence of AAV-specific antibodies. Therefore, it is the intent of the program to provide every patient with the potential for clinical benefit in addition to ensuring patient safety. Uncertainty exists in scaling from the mouse models to humans, but it is known that a higher dose is required in humans to achieve a similar rate of transduction and expression. The three initial candidate doses have been selected based on available nonclinical data and allometric scaling. Based on allometric scaling from mice to humans and the minimal effective dose (MED) of 1.5×10^{11} gc/kg in spf^{ash} mice, a range for the potentially efficacious dose in humans was extrapolated.

The starting dose of 3.0×10^{12} gc/kg was selected to provide evidence of a biological effect in the urea cycle and at least the potential of benefit to patients based on scaling from the efficacy studies in the spf^{ash} mouse. The 1.5×10^{12} gc/kg dose is the lower de-escalation dose and would be implemented should a DLT be observed in the first dosing cohort. This dose represents the lowest dose considered to be potentially efficacious, based on nonclinical data. Lower DTX301 doses would likely only serve to immunize the subject against AAV8. The highest dose of 1.0×10^{13} gc/kg is one-half log higher than the starting dose, which is a standard approach to dose escalation in early phase gene transfer clinical trials, and aligned with the 2015 FDA guidance, "Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products." This dose is limited based on an anticipated 7-fold safety factor from the toxicology study; the high dose, 7.0×10^{13} gc/kg is the anticipated no-observed adverse effect

level (NOAEL) for the study. Further, the first two proposed doses (3×10^{12} gc/kg and 1.5×10^{12} gc/kg) are similar to AAV8 doses that have previously been administered to hemophilia B subjects with reported safety data on clinical outcome measures. Specifically, the doses for other AAV8 gene transfer products administered to humans have included 2×10^{12} vg/kg and 5×10^{12} gc/kg, with no medically meaningful AEs noted. The starting dose for the proposed study is therefore within the range of doses of AAV8 gene transfer that have previously been administered to humans. The range of doses in the planned study have been chosen based on anticipated safety, to allow assessment of pharmacological action, and to offer potential of direct benefit to all subjects.

Studies of AAV and adenovirus vectors in NHPs have been conducted and support the difference between the anticipated profile in humans and the specific safety of the AAV gene therapy vector that is currently in clinical trials across multiple programs today. Monkeys treated with up to 2×10^{12} vg/kg of AAV8-encoding human Factor IX demonstrated no adverse clinical pathology or histological changes. Cynomolgus monkeys injected with up to 5×10^{13} vg/kg of a clinical-grade recombinant (rAAV5-cohPBGD) did not demonstrate any adverse findings following treatment. In contrast, adenovirus administration at the highest dose (1×10^{13} particles/kg) in rhesus monkeys was associated with severe clinical and biochemical toxicity due to hepatitis, similar to that observed in baboons and mice. The differential immune activation from AAV and adenovirus has also been investigated in mice. AAV treatment resulted in transient chemokine and cytokine expression and transient leukocyte recruitment in the livers of mice, with no associated significant toxicity or inflammation. In contrast, the chemokine and cytokine induction following exposure of mice to adenovirus vectors was rapid, marked, and sustained.

Per review and further consideration of the available data, Dimension believes that no new relevant information will be generated by obtaining vaginal swabs or semen as a part of this clinical study. The FDA guidance, “Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products,” states that the types of clinical samples collected to assess shedding should be based on the route of administration of the product, the tropism of the product-based virus or bacteria, and the natural route of transmission and shedding of the parent virus from which the product is derived. AAV8 has previously been administered to humans intravenously and therefore does not represent a novel product. DTX301 is a nonreplicative recombinant AAV (rAAV) vector, so DNA is expected to be present transiently at very low levels in semen, lower than those observed in stool based on data from other AAV vectors. The period of shedding in semen for rAAV vector DNA is expected to be between 1 and 2 months. Moreover, the assays used for shedding measure the presence of vector DNA and do not necessarily represent infectious particles. The protocol specifies the use of barrier contraceptives during the course of the study, including the use of a condom with spermicide for men and, for women, condom with spermicide and one of the following: oral contraceptive, diaphragm or cervical cap, or sterilization. In the unlikely case that infectious particles are shed, partners should be protected. In addition, because DTX301 encodes the normal version of a human protein, no adverse effects would be expected from exposure to low levels of virus.

AAV is a nonpathogenic, non–disease causing vector that carries little to no infection risk. Viral shedding will be carefully monitored throughout the study to ensure that there is no risk of infection. In the unlikely event of transmission to another person, no adverse effects would be expected from exposure to low levels of virus, as DTX301 encodes the normal version of a human protein.

Liver enzymes will be closely monitored, and elevated levels will be confirmed by repeat testing and followed by a thorough investigation to rule out other etiologies by physician investigators with discussions with the sponsor before initiation of oral steroids. While CRS often results in liver enzyme abnormalities, the clinical presentation of this is typically acute, with other signs and symptoms (respiratory distress, acute kidney failure, other organ failure), and requires intensive care hospitalization. In contrast, AAV-induced immune hepatitis is typically asymptomatic in the early stages. The PI will use both the onset and the nature of the symptoms to classify and judge the best course of treatment for each patient exhibiting elevated liver enzymes, which may warrant a course of oral steroids.

Long-term durable expression of the OTC protein after administration of DTX301 is expected based on results from studies in humans and animals. A robust neutralizing antibody response to AAV8 after

administration of DTX301 is also expected, which would preclude re-administration of AAV8 The study team will be monitoring the formation of AAV8 neutralizing antibodies, immunoglobulin G (IgG)–binding antibodies, and T cell–mediated immunity throughout the course of the clinical study as an exploratory endpoint. While the use of alternative serotypes of AAV specifically for the treatment of OTC deficiency is not being assessed now, the investigators recognize the importance of the potential need for re-administration of AAV gene therapies and that this is an active area of interest for the AAV gene therapy community. Dimension is conducting studies with research vectors to determine the potential for re-administration of different serotypes in NHPs.

Decisions on whether to de-escalate, expand, or escalate to the next dosing level after review of all patient data at 6 weeks will be made by Dimension after careful review and consultation with DMC members as per the DMC Charter. The DMC Charter will be agreed upon between the DMC Chair and Dimension prior to the initiation of the study.

Dr. Harding clarified that the proposed study is designed to have a minimum of two and a maximum of four dosing cohorts. Because a continual reassessment model is used, the actual dose escalation process and number of cohorts will be informed by the study data as it develops. For example, a likely scenario is that the first cohort of patients will be dosed with 3×10^{12} gc/kg and then, in the absence of a DLT, the second cohort of patients will be dosed at 1×10^{13} gc/kg. If there is no DLT in the second dose cohort, a third cohort of patients will be dosed with 1×10^{13} gc/kg to confirm that this dose is the optimal biological dose. In another possible scenario, if a DLT is seen in the first dosing cohort at 3×10^{12} gc/kg, the second dosing cohort will receive a lower dose of 1.5×10^{12} gc/kg. If no DLT is seen at 1.5×10^{12} gc/kg, the third dosing cohort would receive a dose of 2×10^{12} gc/kg. Finally, a fourth dosing cohort at 2×10^{12} gc/kg would be enrolled to confirm this OBD.

The protocol will be edited to state that a DLT greater than 25 percent (not greater than or equal) will determine the MTD. Therefore, a DLT in a single patient in the first dosing cohort (n = 3) and subsequent dosing cohorts (n = 2–3) will determine the MTD.

In the proposed clinical study, subjects will initially maintain their ammonia scavenger therapy and their prescribed diet. Starting at week 8, however, ammonia scavenger medications may be gradually tapered at the discretion of the investigator if all of the criteria specified in the protocol are met, including a stable rate of ureagenesis, stable or improved serum ammonium levels, and the subject being clinically stable and asymptomatic. There is no requirement for patients to taper ammonia scavengers or adjust their diet as part of their study participation. A study participant may maintain their prescribed diet and medications throughout the clinical study if that is what their physician recommends based on review of the data.

The protocol, and specifically the plan to taper ammonia scavenger medications, was discussed in detail with members of the Urea Cycle Disorders (UCD) Consortium and the institutional Clinical Advisory Board for this program. Over the course of the study, subjects will be monitored closely and ureagenesis rates will be measured to assess OTC protein functioning and urea cycle kinetics to mitigate any risk associated with medication tapering and diet modification. The withdrawal of ammonia scavenger therapy will only occur with evidence that the pre-specified criteria are met. Some of these medically managed patients may have normal or almost normal 24-hour ammonia levels at baseline. Demonstrating stability or improvement of 24-hour ammonia levels in the setting of tapering ammonia scavenger medications would help provide evidence of a clinically meaningful response to DTX301, but achieving human proof of concept does not depend on medication tapering. By decreasing medication burden, however, patients may experience an improvement in quality of life and better overall treatment compliance. If the patient is clinically stable following the tapering of ammonia scavenger therapy, the subject may be weaned from their low-protein diet at the discretion of the investigator starting at week 12. Such a diet change will be prescribed and managed by the investigator and a dietician. A less restrictive diet could also result in an improvement in quality of life.

The study team acknowledged that Jesse Gelsinger's death was a great tragedy that was very concerning to both the patient and gene therapy communities. Investigations at the time revealed a number of issues related to the preclinical science and also the conduct of the clinical program. The

science around gene therapy has progressed significantly since 1998 with the evolution of capsid technology and the introduction of new capsid serotypes based on the AAV family. These advances have been developed specifically to address the major issues with the previous generation of adenovirus vectors and with patient safety in mind.

The ICD has been clarified to state that clinical efficacy from receiving DTX301 cannot be expected. The ICD has also been revised to provide more information about the inpatient post-infusion observation period, experience with AAV-based gene therapy in other clinical trials, known side effects including vector-induced hepatitis, and how such side effects will be monitored and managed. The ICD has been revised to include the suggested description of the AAV vector that will be used in the proposed trial and differences between the new AAV vector and the adenovirus previously used in OTC deficiency gene therapy trials. A note has been added to indicate that the AAV can infect cells but cannot replicate. The sponsor commented that the recommended text is much clearer than the original language.

The home health care visits will be limited to collection of blood and bodily fluids samples and that other activities, such as measurement of vital signs, will not be done. Such visits are often performed as part of clinical studies with the intent to decrease patient burden. Patients always have the option to come to the clinic for these visits if it is their preference.

2. Responses to RAC Discussion Questions

The investigators acknowledged the variation in ammonia levels across patients and that some patients have chronically elevated ammonia measurements. Dr. Harding agreed that the choice of study sites is important regarding the experience of the investigators and their knowledge of the subjects and in assuring consistency in ammonia measurements across institutions and over the course of the trial. Dr. Eric Crombez, Chief Medical Officer, Dimension Therapeutics, appreciated the comments regarding the ammonia cutoff level. He noted that subjects have the opportunity to be rescreened one time if their ammonia level at the initial screening/baseline visit is high but subsequently resolves; if the inclusion criterion is met at the subsequent screening, the patient may be eligible to enroll. The team will consider the suggestions to lower the same-day cutoff for dosing and take the patient's normal range into account in setting the cutoff.

Dr. Harding noted that infection and concurrent use of steroids has triggered hyperammonemic crises in some of his patients. He pointed out, however, that there are no published data on this complication and that cases to date are anecdotal. It is difficult to know if the event was going to happen because of the primary insult or whether the steroids contributed to the occurrence. Based on available evidence, it is expected that vector-induced hepatitis will be self-limiting. While patients with OTC deficiency are potentially at risk of a hyperammonemic episode if they become infected with wild-type AAV, the investigators were not aware of any data in the literature that wild-type AAV is a trigger for such an event. Whether there is a similar risk with the recombinant virus to be infused into the patients is not known. The proposed trial allows for but does not require treatment with steroids if the subject's ALT increases. The monitoring and treatment plan for use of steroids was developed in collaboration with the UCD Consortium and the local clinical advisory board, which determined that use of steroids is acceptable if the patient is stable and meets the criteria specified in the protocol. ALT levels will be re-measured within 24 hours of the initial test result and, if still elevated, steroid treatment will be initiated, per the clinical investigator's judgment in consultation with the medical monitor. The treating physician will make the decision regarding admission of the patient.

For any AAV, there is an expected age-dependent loss of vector, based on animal studies. The investigators have discussed whether DTX301 could be used as a rescue agent, for example, in sick infants. The current view, however, is that any effect would be temporary. The plan at this point is to investigate the safety and outcomes in adults given this product. Other objectives will need to be addressed in future studies.

Dr. Harding explained that until recently, one of the older nitrogen scavengers had to be taken daily in pill form, causing GI distress and, in turn, often reducing compliance. A newer oil-based version of the

scavenger is better tolerated but not completely without some GI discomfort. Subjects must be on a stable dose of ammonia scavenger therapy for at least 4 weeks prior to study entry. Ammonia scavenger therapy will be monitored at each study visit. Patients can continue use of scavengers over the course of participation but may also taper or ammonia scavengers during the study if it is deemed safe by the investigators.

Dr. Harding has not been directly involved in the AAV8 studies conducted to date. The potential risks of this vector are based largely on published reports of those trials, which indicate a good safety profile with the exception of elevated liver enzymes and transient hepatitis. The elevation in liver enzymes is thought to be due to an immune reaction to the AAV capsid proteins and occurred in hemophilia patients given a high dose of AAV8 (2×10^{12} vg/kg). These transient elevations of liver enzymes occurred between week 7 and 10 weeks after AAV8 vector administration and resolved with 5 days with steroid treatment. Anecdotal reports of administration of much higher doses of AAV per se, including the AAV8 and other capsids, have not identified serious adverse events in nonhuman primates. The doses for the proposed trial will increase using half-log dosing increment, which is the standard recommendation for dose escalation in these types of studies. An additional risk for the planned patient population is the possible triggering of hyperammonemia, as discussed. These responses (transient hepatitis, hyperammonemia) appear to be unique to humans as no similar signals have been seen animal exposed to AAV8, including the spf^{ash} mouse model.

Dr. Sam Wadsworth, Chief Scientific Officer, Dimension Therapeutics, noted three potential sources of toxicity for the proposed study, the excipients in the infusion formulation, which are standard for this research and not expected to be associated with any toxicities; the AAV capsid protein (AAV8 encoding Factor IX) is the same as that used in the Nathwani hemophilia B trials, which showed efficacy and safety of the vector in that population; and the gene product itself and its activity. Thus, the main difference between prior studies and the planned trial is the gene product. The toxicity of the gene product has been tested in the spf^{ash} mouse model, for which levels of orotic acid are used as a biomarker of degree of function or dysfunction of the urea cycle. Expression of the gene product in this model has been reported to be up to five times greater than in wild-type mice, with no adverse events observed in the spf^{ash} mice. Similarly, no adverse events occurred in these animals in association with increased expression of the OTC enzyme (up to 150 higher than in wild-type animals). Results of pre-clinical studies therefore do not anticipate adverse findings in patients.

Two key features of recent studies suggesting increased risk of hepatic cellular carcinoma (HCC) with recombinant AAV vectors are not present in the proposed trial: administration of the vector in the immediate neonatal period, and use of an extremely high vector dose. If either of those two conditions were not present, then the association of HCC with recombinant AAV vectors was not present. Dr. Crombez added that one of the challenges with HCC is there is no simple blood test to test for biomarkers of HCC, making monitoring difficult. Over the course of the proposed trial, however, subjects will be monitored for HCC and other unanticipated adverse events that could occur in these patients.

The DSMB will be informed about the risks of AAV8 and the DTX301. Experience from prior and ongoing clinical trials of AAV8 indicate that patients may have elevated liver enzymes in association with transient hepatitis, the major complication reported to date. While these patients have not been symptomatic, changes in ALT are an important safety signal that will be monitored closely in the planned study. Overexpression of OTC and an immune response to the enzyme encoded in DTX301 are not anticipated in patients enrolled in the proposed trial. To be eligible for the proposed study, patients must, by definition, have late-onset disease and are expected to have some residual enzyme activity.

Dr. Wadsworth explained that the starting dose for the proposed trial (3.0×10^{12} gc/kg) was selected on the basis of the minimally efficacious dose identified in preclinical studies of the spf^{ash} mouse model for OTC deficiency. A scaling factor was then applied to this preclinical dose to adjust for human use; per available literature, the mouse-to-human scaling factor is between 10 and 40, so that the human dose is 10–40 times higher than that for the mouse. The dose at the low end of that scale is 1.5×10^{12} gc/kg, which the investigators believe is probably the lowest dose for which a pharmacological response in this patient population will be observed, in accordance with FDA guidance for Phase I and 2 cell and gene

therapy protocols. A lower dose would most likely simply immunize patients against AAV8, which would preclude them for receiving another dose. The dose at the high end of the scale is 6.0×10^{12} gc/kg. The starting dose is in the middle range between the low and high scaled doses and is intended to be safe. The highest planned dose for the proposed trial is 1.0×10^{13} gc/kg. Results of ongoing GLP toxicology studies will further inform the safety aspects of the protocol, including dose selection and plans for dose escalation or de-escalation.

The investigators agreed with the reviewers that one of the biggest challenges with drug development for rare diseases is the relatively small pool of patients. Use of an adaptive design and a continuous reassessment model allows for incorporation of data from the first dosing cohort to determine the next dosing cohort. With this approach, fewer subjects are needed at a lower that may not be optimal, thereby making it possible to proceed more quickly and efficiently to determine the optimal biologic dose.

The protocol follows FDA guidelines for collection of samples for viral shedding and will analyze blood, urine, and saliva samples as surrogates for overall excretion of the viral vector. Some work shows that AAV clears more quickly from on other bodily fluids than from the three specimens that will be collected. The investigators are confident that the proposed sample collection and analysis plan is appropriate and sufficient to document clearance of AAV8. There are no plans to collect semen or vaginal samples.

The criteria for the DLT will be clarified to reflect a whole number in terms of affected subjects, not a fraction of a subject (e.g., 33 percent based on three subjects per cohort instead of 25 percent of three subjects per cohort). A patient who has a hyperammonemic event that was clearly related to a factor other than administration of the vector would not immediately stop the trial. In contrast, if the investigators determine that the hyperammonemia was related to the intervention, that event would meet the stopping criteria. If a patient has recurrent episodes, the investigators would need to carefully assess the relationship between the events and the study drug before determining whether the events should result in stopping the study. The timing of the adverse event in relation to administration of DTX301 would be a critical factor in making these determinations.

Dr. Harding noted that the spf^{ash} mouse model is very analogous to partial OTC deficiency in humans. The animals do not have chronically elevated ammonia levels unless they are challenged with ammonia or a high-protein diet. Preclinical data indicate that DTX301 and the AAV8 vector are safe. Groups other than the OHSU team have studied AAV8 gene therapy in spf^{ash} mice with very similar results. In preclinical studies of DTX301, baseline ammonia levels in the sparse fur mice were not elevated, and animals given the highest dose of the investigational product tested (1.5×10^{12} gc/kg, equivalent to 3.0×10^{10} gc/mouse) had nearly complete protection against an ammonia challenge.

Regarding efficiency of the transgene, Dr. Harding explained that if transgene expression is confined to a small group of cells (e.g., 3–4 percent), it is unlikely that the problem will be corrected, even with very high levels of enzyme per cell. The exact percentage of cells that would be needed to be transduced to overcome the enzyme deficiency is difficult to estimate. The best data have been extrapolated from human females with OTC deficiency, who are mosaics (i.e., they have one X chromosome with normal expression in some cells, while the other X chromosome is inactive and produces little or no enzyme). Based on extrapolation of these data, 10–20 percent of hepatocytes would need to be transduced to achieve a relatively normal level of ureagenesis. The percentage of hepatocytes transduced at the lowest dose tested in the sparse fur mouse model is also in this range. Differences in the anatomy and physiology of the mouse, NHP, and human liver affect the outcome of gene transfer. For example, the most abundant gene transfer in the mouse is around the central vein, which is not the portion of the liver involved in the urea cycle (i.e., around the periportal region). In contrast, there is more gene transfer around the periportal region in NHPs and presumably in humans, but this also reflects an extrapolation. The transduced cells do not appear to have a survival advantage over non-transduced cells in the liver.

Studies in relevant animal models have consistently predicted the risk of medically significant safety-related AEs associated with the administration of AAV and adenovirus vectors. Mice have been judged to be an appropriate species to investigate the potential toxicity of AAV administration as the tropism to the liver is expected to be similar and the transduction efficiency is much higher than in humans. Therefore,

Dr. Harding noted, an NHP study is not expected to provide additional safety information for DTX301 beyond that obtained from the proposed nonclinical studies in mice. He added that safety studies in support of the IND for this program were designed in consultation with the FDA, which did not recommend that the team conduct nonhuman primate studies in preparation for human dosing in patients with OTC deficiency. With the goal to reduce animal use, Dimension has proposed and reached agreements with several health authorities, including the FDA and Health Canada, to conduct studies in mice to investigate the potential safety profile of DTX301 in humans.

Dr. Harding pointed out, as did the reviewers and other members of the RAC, that DTX301 is a different product made with a different vector that has been shown to be generally safe following administration to several hundred human subjects in the context of clinical research programs. The investigators feel that it is appropriate to include in the consent form only that information that is specific to the proposed clinical trial and that informs the patient about the experimental nature of the current product and the known and potentially unknown risks of IV administration based on preclinical data specific to DTX301 and relevant related programs of AAV therapy in the field. The study team understands that patients may have questions related to the previous trial during the informed consent process. The investigators on this trial have been carefully selected based on their specific medical training and experience treating patients with this disease and their experience in conducting clinical trials.

Comments regarding the controversies and setbacks following Jesse Gelsinger's death were acknowledged. The investigators agree with the RAC that this research program and the proposed protocol may have increased media attention due to the history of gene therapy for this indication. The team is dedicated to not repeat the mistakes made in the prior trial and has have taken specific steps to ensure strong collaboration with the treating specialists and relevant patient associations in the United States, Europe, and Canada to ensure that the approach to the design and conduct of the clinical program is compliant with all relevant standards.

Dr. Harding clarified that the proposed trial will be open to patients who are struggling with adherence to their therapy and are not completely asymptomatic. Subjects must have had at least one hyperammonemic episode to be eligible to participate. He acknowledged that selecting the most appropriate cohort involves balancing between healthier versus sicker patients and assessing the risks and possible benefit for the patients who enroll. One concern with enrolling patients who have more severe, symptomatic illness is that it likely would be difficult to differentiate between a safety signal and the patient's disease.

Due to the formation of anti-AAV8 antibodies that are expected after the administration of DTX301, re-administration of DTX301 or any other AAV8 gene therapy is precluded. Although the primary goal of the proposed Phase I/II clinical trial is safety, it is the position of the sponsor that all study subjects are offered a potentially pharmacologically active dose based on available preclinical data. Because of the expectation that study subjects will develop neutralizing antibodies to AAV8 after initial administration of DTX301, efficacy and overall benefit-risk profile considerations are also included in the dosing rationale, as detailed in the protocol.

Dr. Crombez clarified that patients will not necessarily need to come to the study site for all monitoring visits and evaluations. Those who live close to the study site and are able to travel could do so, but patients can also have monitoring done by their treating physician. The investigators have been working with the UCD Consortium to establish collaborations with eight centers (thus far) across the Unites States that have and the highest concentration of patients and experts in this field. Through these collaborations, the local providers are able to remain up to date on upcoming and ongoing trials and guidance for clinical care.

Dr. Wadsworth thanked the RAC members for their diligent work in reviewing the protocol and in providing their feedback and recommendations. However, he wanted to make a statement for the record on behalf of Dimension Therapeutics that the sponsor does not believe, based on all available scientific data, that carrying out NHP studies will add to the safety assessment of DTX301. This position is based on several factors. First, healthy nonhuman primates do not represent in any way the physiology of a

human subject with OTC deficiency. Thus, there would be very little benefit in conducting those studies. Second, the team has done extensive safety testing in mice in an animal model for OTC deficient, and per the literature, if the proper studies are done in mice, it is easy to distinguish the toxicity of an adeno-virus vector versus the effects of recombinant AAV vectors, which are minimal to none in this species. Safety studies are ongoing in this mouse model. Third, an additional safety study (developed per consultation with FDA) is underway in both the relevant animal model of the disease and in healthy C57 black mice. Fourth, the events in the UPenn trial occurred nearly 20 years ago, and there has been a great deal of technology advancement in the interim. In addition, hundreds of human subjects with various medical conditions have been exposed to a variety of recombinant AAV vectors produced using different methods. Results in these subjects indicate a very good safety profile for recombinant AAV in humans, with the only finding being transient self-limiting increases in liver enzymes.

E. Public Comment

Dr. Gavin clarified that, as a general rule, the FDA does not always require two species for safety assessment. The agency recently finalized the pharmacology-toxicology guidance for early-phase clinical studies of gene therapy and cell therapies; the guidance includes a discussion about having an applicable animal model, which might preclude testing in more than one species. Whether one or two species are required would be evaluated on case-by-case basis.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

<u>Clinical and Trial Design</u>

- There should be harmonization of dosing regimens in the preclinical toxicity studies in two animal species at the highest dose anticipated to be used in humans prior to moving forward to human studies.
    - After safety has been demonstrated in appropriate animal models, lowering the dose to a half log lower than any AAV8 dose used in a human as well as appropriate animal models should be considered.
- On the day of entry, the ammonia level should not exceed the upper limit of normal for that research participant at that institution and certainly be <100 µmol/L.
- Elevated liver function tests should be followed medically in the absence of clinical symptoms as corticosteroid administration can have deleterious consequences. If steroid therapy is required, hospitalization should be strongly considered.
- Please correct language regarding dose-limiting toxicities greater than or equal to 25 percent of dosing cohort, given that only two to three research participants are enrolled per cohort.
- Amend the research participant population to include research participants who are not doing well on current therapies.
- Suggest ensuring IRBs are able to review all points in Appendix M.

<u>Ethical, Legal, and Social Issues</u>

- The RAC strongly urges focusing on safety issues rather than efficacy at this stage in communicating the goals of the study to local officials and research participants. Clarify that there is no anticipation of benefit in this Phase I study.
- Regarding terminology, please change "study doctors" to "investigators," and "gene therapy" to "gene transfer protocol."
- Please add "death" as a risk of the study. Add information about the previous death of Jesse Gelsinger in the informed consent document, and explain why this study is different from that previous case.

- Ensure the language in the informed consent document is technically correct and understandable.
- Consider adding to the Data Safety Monitoring Board an ethicist(s) and other community perspectives outside of scientists and clinicians.

G. Committee Motion 4

Dr. Whitley summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Whitley requested a vote, and the RAC approved these summarized recommendations by a vote of 11 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VIII. Day 1 Adjournment

Dr. Whitley adjourned Day 1 of the June 2016 RAC meeting at 4:45 p.m. on June 21.

IX. Day 2 Opening

Dr. Whitley opened Day 2 of the June 2016 RAC meeting at 8:30 a.m. on June 22.

X. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Atkins, Curry, Donahue, Kaufman, Kiem, Lee, Pilewski, and Whitley

A. GTSAB Report

Dr. Whitley opened the session by reviewing the charge to the GTSAB:

- Review in closed session, as appropriate, safety information from gene transfer trials for the purpose of assessing toxicity and safety data across gene transfer trials.
- Identify significant trends or significant single events.
- Report significant findings and aggregated trend data to the RAC and thereby disseminate it to the scientific and patient communities and to the general public.

The GTSAB review process enhances review of new protocols; improves the development, design, and conduct of human gene transfer trials; promotes public understanding and awareness of the safety of human gene transfer research studies; and informs decision-making of potential research participants.

The current GTSAB roster includes eight RAC members and two FDA representatives.

Dr. Whitley then presented the GTSAB report for the second quarter of 2016. Within the past 3 months, OSP received a total of 26 protocol submissions, 22 of which were not selected for public review at this RAC meeting. Five protocols were selected for public review at the current RAC meeting, including one protocol that was deferred in the prior quarter. Of the 22 protocols not selected for public review, 19 were oncology protocols, 1 was a monogenic disease protocol, 1 was an infectious disease protocol, and 1 was a peripheral artery disease protocol. Among these 22 protocols, 5 used lentivirus, 4 used retroviruses, 4 used plasmid, 3 used vaccinia virus/fowlpox, 2 used herpes simplex virus, 1 used adenovirus, 1 used AAV, 1 used *Listeria,* and 1 used RNA.

For the second quarter of 2016, the GTSAB reviewed 24 serious adverse events (SAEs) (initial and follow-up reports)from 15 protocols. In the future, it will be available in the NIH Genetic Modification Clinical Research Information System, also known as GeMCRIS.)

Dr. Whitley provided an update from the last GTSAB meeting regarding an SAE involving delayed listeriosis in a Phase II trial (Protocol 1082) evaluating AVXS11-001, an attenuated *Listeria*

monocytogenes (Lm) vaccine strain for the treatment of persistent or recurring squamous cell or non–squamous cell carcinoma of the cervix. In response to this SAE, the protocol was further amended to:

- Add new and prolonged antibiotic regimens for eradication of the attenuated *Lm* strain.
- Add blood cultures for monitoring persistent *Lm* infections.
- Exclude subjects with implants and hardware that are not easily removable.
- Add potential risks of delayed listeriosis to the Investigators Brochure and the Informed Consent Document (ICD).
- Exclude subjects who have used certain immunosuppressive agents.
- Extend follow-up of study participants for delayed listeriosis to 3 years.

In response to recent SAEs involving prolonged pancytopenia in a Phase I protocol (Protocol 1071), the investigators have amended the protocol to evaluate alternative lymphodepletion regimens, including having cohorts with high tumor antigen expression receive lymphodepletion chemotherapy with only cyclophosphamide or a reduced dose of fludarabine and cyclophosphamide.

Several additional SAEs have been reported in a single Phase II CD19 CAR T-cell trial (Protocol1339). These events involved severe cytokine release syndrome (CRS) and neurotoxicity, including confusion and seizures. One SAE involved CRS and early neurotoxicity complicated by multiple lab abnormalities and subsequent cardiac arrest. Another SAE involved a Grade 3 neurotoxicity complicated by multiple lab abnormalities and, eventually, hemophagocytic lymphohistiocytosis. An additional SAE involved a Grade 4 neurotoxicity complicated by leukoencephalopathy on central nervous system (CNS) imaging and extremity weakness.

Most of the other SAEs are from CAR and TCR studies, as described below. Similar events, including signs and symptoms of CRS, were seen across different protocols:

- **CD19 CAR T cell trial targeting B-cell lymphoma** (Protocol 940): This trial met the criteria for stopping rules due to toxicities. As per the principal investigator (PI), neurotoxicity was the most important toxicity experienced by subjects in this study.
- **CD19 CAR T cell trial against B-ALL** (Protocol 1351): This Phase II trial reported an SAE involving severe CRS complicated by hypernatremia and later by an encephalopathy of unclear etiology.
- **CD19 CAR T cell trial** (Protocol1419): This Phase II trial reported an SAE with severe CRS, which was later complicated by encephalopathy, seizures, cerebral edema, and severe hypernatremia.

For the second quarter of 2016, OSP received notification that 15 new protocols opened, two of which were publicly reviewed:

- **Protocol 1404-1307:** A Phase I Clinical Trial of Cyclophosphamide Followed by Intravenous and Intraperitoneal Infusion of Autologous T Cells Genetically Engineered to Secrete IL-12 and to Target the MUCI6ecto Antigen in Patients with Recurrent MUC16ecto+ Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer
- **Protocol 1508-1455:** The Effect of Vorinostat and AGS-004 on Persistent HIV-1 Infection (The VOR VAX Study)

Recent publications and reports related to OSP protocols are summarized below:

- An article posted on Medscape, “CAR T-Cell Therapy: Getting a Handle on Toxicity,” reports that “chimeric antigen receptor (CAR) T-cell therapies are causing a stir in leukemia and lymphoma circles, with impressive response rates and sustained remission in patients with advanced, chemorefractive disease.” According to Medscape, “With more than 20 American trials looking at the anti-CD19 CAR T-cell space, treatment toxicity is emerging as a major focus.” Catherine Bollard, MD, who “acted as discussant for a series of three studies on CAR-CD19 T-cell therapy” at the ASCO Annual Meeting, said, “There is no gain without pain, and the ‘cytokine release syndrome’ [CRS] does remain a problem, although multiple groups are looking at ways of preventing it.” Medscape points out that “in one study...James Kochenderfer, MD, from the Experimental Transplantation and Immunology Branch of the National Cancer Institute, showed

that lightening up on pretreatment conditioning chemotherapy can cut toxicity without sacrificing efficacy."

- The first gene therapy for children and second gene therapy in Europe (after Glybera, which was approved in 2014), was approved in late May 2016, for children with adenosine deaminase deficiency-severe combined immunodeficiency (ADA-SCID). The product (Strimvelis) was developed by investigators at Vita-Salute San Raffaele University in collaboration with the Italian biotechnology company MolMed. GSK licensed the investigational product, which involves autologous CD34+ cells transduced to express the *ADA* gene, in 2010.
- Pro and con positions have been published in response to the Nault et al., article, "Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas" (*Nat Genet* 47:1187–1193, 2015). One was a commentary that stated, "Close follow-ups of patients treated with AAV vectors will shed light on some of these issues, and renewed research into the potential oncogenicity of AAV vectors is now more important than ever."

Dr. Whitely thanked the OSP staff for compiling information for the GTSAB. To learn more about the Office of Science Policy, including RAC meeting updates, committee members were invited to visit the OSP blog, "Under the Poliscope," at http://osp.od.nih.gov/under-the-poliscope. General inquiries can be posted to SciencePolicy@od.nih.gov.

B. RAC Discussion

Dr. Zoloth inquired about the differences in the responses to the report of AAV2-related insertional mutagenesis in hepatocellular carcinoma. Dr. Whitley explained that the commentary, "AAV2 and Hepatocellular Carcinoma," appeared earlier this year in *Human Gene Therapy* in response to the paper by Nault et al. One response by Berns et al. refuted the findings of the original study Nault and colleagues. The other was a study that agreed with the conclusions of Nault et al. and was reported by Russell and Grompe in an editorial in response to the *Human Gene Therapy* article. The finding of AAV2-related insertional mutagenesis in hepatocellular carcinoma is something the RAC should be aware of and monitor going forward.

Drs. Atkins and Cho requested further information regarding the report of a death as an SAE for CAR T cells. Dr. Whitley noted that the death in Protocol 1339 was from cardiac arrest. Two additional deaths were also reported in the last quarter (both on protocol1339). Of these two additional deaths, one was possibly related to the investigational product. The more consistent findings with CAR T cells are neurotoxicity, lymphopenia, and CRS in addition to a few cases of hypernatremia.

Drs. Wooley and Zoloth inquired about a publicly accessible database for the NIH/OSP protocols trials and whether such a resource would include an accumulated summary of all deaths on these protocols. All data regarding NIH/OSP registered gene transfer trials are contained in the Genetic Modification Clinical Research Informaiton System (GeMCRIS®) database. Data are compiled quarterly and per discussions with the GTSAB. GeMCRIS is a relational database that includes outcomes and events of all types and Grades. Staff can provide an overview of those outcomes, including deaths, by querying the database.

C. Public Comment

No public comments were offered.

XI. Review and Discussion of Human Gene Transfer Protocol #1603-1512: Phase I Study of Oncolytic Polio/Rhinovirus Recombinant against Recurrent Malignant Glioma in Children

Presenters: Matheus Gromeier, M.D., Duke University

RAC Reviewers: Drs. Cho, Curry, and Kaufman

Dr. Whitley was recused from consideration of this protocol due to a conflict of interest. As a result of Dr. Whitley's recusal, Dr. Curry chaired this section of the June 2016 RAC meeting.

A. Protocol Summary

Pediatric brain tumors are the most common solid tumor in children with approximately 4,000 new cases every year in the United States. Although significant improvements have been made in the treatment of these children, the outcome for those with recurrent brain tumors remains grim, particularly for those with recurrent malignant glioma. Treatment failure is frequently due to poor penetration of cytotoxic drugs into areas where the blood-brain barrier is intact and poor tumor control. Various approaches have been used successfully to circumvent the blood-tumor barrier, including convection-enhanced delivery (CED), a process by which large molecules (>400 daltons) are directly infused under pressure into a tumor through a catheter. CED results in adequate distribution of such molecules into the tumor over large areas via inherent interstitial fluid pathways. Vector-directed tumor cell lysis has been previously employed for the intervention of various tumor types using different attenuated replication-competent viruses.

Oncolytic virus immunotherapy for brain tumors is a unique approach with several advantages over more conventional drugs. Certain oncolytic viruses are capable of selective tumor cell killing with a range of inflammatory and immunostimulatory effects on the tumor itself, the tumor stromal component, and the host immune system at large. The objective of oncolytic immunotherapy is to recruit effector adaptive immune responses against tumor-associated antigens that can produce lasting immunologic control of cancers.

The investigators have developed a recombinant polio virus–rhinovirus chimera from the genome of a live attenuated poliovirus (PV) serotype 1 (Sabin) vaccine with the internal ribosome entry site (IRES) of human rhinovirus serotype 2. This chimeric virus product, PVSRIPO, retains the ability to infect cells that express the PV receptor, CD155, which is broadly expressed on cancerous cells, cancer "stem-cell-like cells," and tumor-associated proliferating vasculature. PV's inherent neuropathogenicity has been removed by the IRES exchange, which ablated the virus's ability to propagate in cells of neuronal lineage and to cause poliomyelitis. Infection with PVSRIPO results in swift destruction of tumor cells. The modified viral agent replicates efficiently in cancerous cells and exhibits potent anti-neoplastic effects in animal tumor models. Tumor cell-specificity is mediated by the foreign IRES in PVSRIPO.

PVSRIPO is being tested in a Phase I trial of adults with histologically-confirmed recurrent Stage IV malignant glioma; this trial includes five dose levels of PVSRIPO ranging from 1×10^8 to 1×10^{10} (50 percent of the tissue culture infectious dose [$TCID_{50}$]) (NIH protocol #0504-707). As of December 2015, four patients were alive at 15 to 43 months post-infusion; three of these research participants received dose levels 1 and 2, and one received dose level 5 but had an intracranial hemorrhage after catheter removal. A single DLT and post-dosing inflammation requiring prolonged use of steroids and other agents (e.g., bevacizumab) led to de-escalation to a dose of 5×10^7 $TCID_{50}$. Eighteen patients have been treated at the lowered dose (5×10^7 $TCID_{50}$). As of December 2015, 15 research participants given this dose were alive; the longest post-infusion interval for this group was 13.9 months, with six of the participants at up to 6 months post-infusion.

The proposed Phase I trial, as submitted for review, will use a standard (3+3) dose-escalation design to assess a single infusion of PVSRIPO at two doses (1×10^7 and 5×10^7) in up to 12 children age 12 or older but less than 18 with recurrent Grade III or IV malignant glioma. As presented and discussed during the meeting, a different design that includes younger children and a starting dose of 1×10^7 may be followed for this trial; specifically, if there are no safety concerns per FDA in the first six subjects, the study will proceed to enroll an additional six research participants age 5–12. The primary objective is to determine the optimum dose when delivered intracerebrally using CED. The secondary objective is to estimate overall survival. The Duke University Safety Oversight Committee and an independent, external DSMB-Plus will be used to monitor safety.

B. Written Reviews by RAC Members

Six RAC members made a recommendation that this protocol should be undergo in-depth review and public discussion. The proposed trial was found to warrant public review because of the combination of the DLT in the trial of PVSRIPO in adults and the special population (children) that will be enrolled.

Three RAC members provided written reviews of this Phase I protocol.

The reviewers noted the researchers' experience in the development and use of the investigational virus and in the treatment of patients with recurrent malignant gliomas. The Duke Brain Tumor Center is one of the leading sites dedicated to the development of oncolytic viruses for the treatment of brain tumors. The high incidence of and very poor outcome for gliomas in children, lack of effective therapeutic options for this population, and strong experience of this academic group support the proposed clinical research study in pediatric patients.

The investigators have adjusted the proposed dose for this trial based upon their prior experience in the adult trial--using lower doses of PVSRIPO to avoid dose-limiting toxicity. Dr. Cho noted, however, there is not much post-infusion information yet regarding the outcomes for the second cohort of 18 adult patients, who received the lower de-escalated dose in the ongoing trial. Additional information is needed as to whether tumor inflammation has been observed in the adult patients receiving the lower dose and whether any of these subjects have required steroid treatment. In addition, she asked how the patients have responded to bevacizumab (Avastin).

Drs. Cho and Kaufman commented on the overly technical and complex content in the assent form, which is nearly identical to the consent document. The assent form needs considerable modification so that it is at a reading level that is understandable to the children and adolescents who will enroll in the protocol. Statements about withdrawing from the study need to clearly indicate that it might be impossible to reverse the effects of the infusion. In the discussion of benefits, the consent document should state clearly that the main purpose of the study is to assess toxicity, not effectiveness, and "possible benefits" should not be listed, as they are unlikely.

Dr. Curry noted that PVSRIPO has long been in preclinical and clinical development and that the work regarding the engineering, preclinical efficacy, toxicity, and early clinical trials of this agent has been rigorously executed to date. The novelty with the proposed research is the use of the agent in pediatric subjects. The history in adult patients helps to assess the doses chosen for the trial.

Dr. Curry had the following additional comments and questions for the investigators:

- One of the eligibility criteria is that subjects must have a recurrent tumor between 1 cm and 5.5 cm. It is not clear whether this figure refers to the greatest diameter or volume of the tumor.
- Two tables in the protocol indicate per their titles ("Toxicity and outcome…") that they are intended to describe the clinical responses in a subset of adult glioblastoma patients in the ongoing trial of PVSRIPO. The toxicities are not actually described in the tables, however. For example, the fact that corticosteroids were required, presumably as a result of cerebral edema, is not included in the tables.
- What are the largest tumor volumes treated to date, and what have been the outcomes for these patients?
- It would be very useful to have updated data about the toxicity outcomes in the 15 research participants given the de-escalated dose of PVSRIPO. Nine of these subjects with at least 2 months of follow-up started Avastin (two subjects without Avastin died relatively early). Is Avastin therapy so common because of treatment-related edema, and how has that been differentiated from progression? How soon after infusion of PVSRIPO is cerebral edema typically observed, particularly in participants enrolled at dose level 1? What is the range of time points at which symptomatic cerebral edema has been identified?
- Regarding the definition of DLTs, special attention is needed because of some of the unique features, particularly the wording, "new or worsening neurologic deficits." The protocol states that "a new neurological deficit, which resolves within 2 weeks after initiation of medical therapy, will not be considered a DLT." Does the deficit have to resolve fully, that is, back to pretreatment baseline to meet this criterion? Medical therapy presumably includes corticosteroids. Does

"medical therapy" also include Avastin, which typically cannot be started until 4 weeks after a surgical procedure? These provisions and questions also relate to the "Special Consideration" section of the protocol, in consideration of cerebral edema and increased intracranial pressure. If a perceived inflammatory reaction requiring 4 mg of dexamethasone after day 14 occurs, participants will be treated with IV bevacizumab, and steroids will be reduced.

- It is not clear how a relative contraindication to bevacizumab, such as an intratumoral hemorrhage, will be classified, particularly if steroids prove insufficient. Would this type of event be considered a DLT?
- A related concern about the study design is that it may demonstrate safety in the population that can subsequently be treated with bevacizumab, which will confound radiographic analyses and progression data. Dr. Curry asked the investigators to comment on this point, especially with regard to the findings presented for the subset of adult glioblastoma patients in the ongoing trial of PVSRIPO.

Dr. Kaufman had the following comments and questions about the clinical protocol and the ICD:

- The plans to administer a single dose of virus given the complexities of accessing the CNS are clear. Other oncolytic viruses, however, have required multiple dosing. Data to support the single dosing regimen should be provided.
- The major toxicity appears to be intracerebral inflammation. The trial will not count neurologic sequelae that are "reasonably attributed to disease progression." How will the investigators distinguish disease-related from treatment-related neurotoxicity?
- The investigators discuss the low risk of transmission to household contacts but did not mention the potential risk of transmission to health care providers. Are there education programs and/or standard operating procedures (SOPs) in place that will cover pharmacists, nurses, pediatricians, radiologists, house staff, and operating room personnel? Is polio vaccination required? How will potential exposures be managed?
- The consent form states, "This study will also look at whether the study drug kills glioma cells and reduces tumor size and whether that improves survival." This study is not powered to look at a survival benefit but only to record overall survival; therefore, this statement is misleading and should be revised to reflect the actual study outcomes.
- The description of general anesthesia in the consent needs to be corrected from saying "general anesthesia (numbing medicine)" to explaining that general anesthesia puts patients to sleep, in contrast with local anesthesia, which is numbing.
- The ICD should mention alternative treatment approaches, including not pursuing further therapy and/or hospice care.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed. They went through their comments and the investigators' responses to their queries and suggestions.
- Several questions were raised regarding treatment- vs. disease-related cerebral edema, how these conditions are distinguished, and how edema is managed (with steroids and/or Avastin). Further clarification and detail are needed as to whether the treatment-associated edema in the trial of adult patients was dose-dependent. Specifically, what were the steroid requirements of subjects treated with dose levels 1 and 2, and was there any neurological decline among these patients? Even though the number of patients given the study agent is small, the results do not initially appear to indicate that the disease response is dose dependent. It is not clear whether the cerebral edema and the need for steroids or Avastin are related to the volume of the tumor.
- Dr. Zoloth noted that the statement in the consent about the possible benefits of participation seems coercive ("Possible benefits of your participation include possible improvement in the symptoms of your disease, delayed growth of your tumor, and/or lengthening the time of your survival, but this cannot be guaranteed."). It should be rewritten to remove any perception of coercion and to make it clear that participation is voluntary.

- Dr. Ross inquired about the risk of wound healing related to the study procedure, which involves catheterization for delivery of the investigational product.
- Dr. Cho found the rationale for dose de-escalation to be compelling, but requested additional information regarding the first group of research participants given the "minus 1" dose of PVSRIPO (5×10^7), specifically, if there were any notable differences between participants who died versus those who survived post-intervention. For example, did any of the participants who died received Avastin? She noted that the two complete responses in this cohort were both participants that had subtotal resections versus gross total resection and asked about the impact of this difference on their recovery. Dr. Curry similarly asked whether this factor would be relevant in the pediatric protocol because it appears that the pediatric patients will not undergo resection, only stereotactic biopsy. Dr. Cho also asked why some data under the "survival" column in the table shown during the presentation are enclosed in a red-outlined box.
- Dr. Curry asked whether the brisk inflammatory responses, which were observed on radiographs, were also detected at the time of stereotactic biopsy as well as with analysis of autopsy specimens. He also requested additional information about the relationship between the size or the volume of the tumor and either toxicity or response, whether Avastin is effective in counteracting inflammation, and how this drug impacts outcomes of the investigational product. In addition, Dr. Curry inquired as to why Avastin is not written into the protocol.
- Dr. Atkins inquired about the role of lomustine (CCNU) chemotherapy on patient outcomes, given the variable results reported thus far with this regimen, including two patients who went into complete remission with this treatment, one of which also received Avastin. Additional information on the underlying mechanism by which this regimen works (e.g., on regulator T cells [Tregs] or *PD-1* expression) could be informative with respect to identifying a more selective way of targeting the tumors.
- Dr. Pilewski requested additional information as to what has been learned from the distribution studies using gadolinium contrast, specifically, whether there is any correlation between how the infusate distributes and the response of individual patients.
- Dr. Atkins suggested that a system be put in place to triage potential participants for the trial in anticipation of considerable interest in this research.
- Dr. Cho noted that the preferred approach before enrolling pediatric patients in a clinical trial is to test the investigational product first in adults, including dose-escalation studies. Since these studies have not yet been completed, what dose will be given to children, and how confident are the investigators in the available data to proceed as planned? Dr. Cho also asked the investigators to clarify whether they plan to test lower doses in the adults and children concurrently or sequentially.
- Drs. Atkins and Curry requested clarification as to the study design, specifically, whether the plan is to proceed with a dose-escalation trial in the pediatric cohort, or whether the study will stop if the first three patients tolerate the investigational product. Upon further discussion with the investigators, it was noted that the trial needs to be revised to include the details of the revised study design, which were not delineated in the protocol submitted to the RAC for review.
- Dr. Atkins suggested rewriting the protocol as a Phase Ia/b trial with efficacy as a secondary goal. Although PVSRIPO has not yet been tested in children, he did not agree with the view the intervention is completely experimental at this stage since PVSRIPO has already been given to a number of participants, some of whom have shown clinical benefit. Thus, to say that safety assessment is the only purpose of the proposed trial is not accurate. Dr. Cho appreciated these points but noted that the trial will be a first-in-child study of PVSRIPO, and not simply an extension of testing in adults. Before proceeding to a trial in pediatric patients (if possible), however, safety of the investigational product needs to be established in adults, along with any data on efficacy. Dr. Cho was concerned that the research is still in the stage where the safety of PVSRIPO at different dosing levels is being assessed, and that the lowest effective dose has not yet been determined. Intracerebral edema that requires Avastin has been reported in more than half of the patients treated thus far, even at the minus-1 dose level. It therefore seems reasonable to test the next lower dose in adults to assess the safety profile at that level to be confident in the dose in the adult setting before moving on to the pediatric patients. Dr. Zoloth agreed, noting that at best, it is only a hypothesis that the planned starting dose in children is acceptable. Children

are not small adults, however, and the de-escalated adult dose may not be the lowest dose that can achieve the desired results in the pediatric population. She further stressed that if a triage system is implemented, it should be a rational plan that is based on whether an individual (or their family) has insurance coverage or is able to pay for whatever costs might be incurred with participation.

- Dr. Zoloth found the presentation to be very clear and the risk-benefit section of the consent to be a model for Phase I trials. She suggested some minor changes in the consent language to clarify certain aspects of this research study.
    - The word "investigator" should be used instead of "study doctor" and “subject” instead of “patient” to better convey the nature of this relationship, which is between the investigator and subject, not between a patient and the study doctor.
    - The consent document needs to state clearly that the intervention is experimental or investigational so as not to imply that PVSRIPO is a “treatment” with demonstrated efficacy.
    - Use of the term “drug” should also be re-considered and perhaps replace with the phrase “modified virus,” which describes what the agent is. Dr. Zoloth did not agree with the investigators’ suggestion to refer to the product as a vaccine.
    - Language describing the goal of the study could be strengthened by saying that the investigators are looking for toxicities associated with PVSRIPO in pediatric patients.
    - A brief description of the adult studies should be included near the front of the document so that patients and their parents know that PVSRIPO has been tested in humans and that the proposed trial is the next phase of this research.
    - The alternatives section of the ICD clearly describes treatment options and options for supportive care. The statement about hospice care “with or without treatment” is confusing, however, and needs to be clarified, including whether hospice would be available through the trial. Further clarification is also needed regarding “combinations of [other] drugs,” especially if these other treatments are not available.
    - A picture or some type of graphic depicting what is involved in delivery of PVSRIPO (e.g., having a hole drilled into the skull, use of the catheter, etc.) should be included with the consent and assent documents so that the subjects have a better understanding of what they are being asked to agree to.
- Statements about and provisions for coverage of the costs of experimental tests and procedures need to be reconsidered. The phrase, "how much you will have to pay depends on whether or not you have insurance and what costs your insurance will cover," is misleading at best because in fact, it is very unlikely that any insurance provider will ever cover experimental care. As with other protocols the RAC has reviewed, such provisions are likely to exclude those who cannot pay because they do not have insurance and/or are poor. Dr. Zoloth noted the trend over the past few years in providing less and less support for subjects who participate in these important and very arduous studies. She stated that the costs for housing, travel, and any additional tests (including clinical tests and exams) should be a covered benefit for participating in the study and should not be imposed on the subject or their family.
- The investigators need to assure that the consent clearly states that the purpose of the study is to assess toxicity, not effectiveness, and that it is unlikely or very unlikely that patients will benefit directly from participating in the trial. In addition, statements about withdrawing from the study need to clarify that the investigational product cannot be removed once it is administered, that it might be impossible to reverse the effects of the infusion, and that “withdrawing” means that subjects will no longer be followed.
- Drs. Cho and Zoloth noted that a mechanism for assenting the pediatric patients is needed, including a separate assent form (or forms) written in language that is age-appropriate.
- Dr. Kaufman had a follow-up question about the specific procedures in place to protect healthcare workers against accidental injection of the investigational product (e.g., education/training, polio vaccination prior to working on the study and handling the agent). Dr. Zoloth added that provisions should be in place ensure that family members are vaccinated and that they are educated about possible risks and any safety measures to follow.

D. Investigator Response

1. Written Responses to RAC Reviews

The use of PVSRIPO is governed by a guidance document that is similar to an SOP and is called the Institutional Product Handling Plan (IPHP). The IPHP was crafted in response to prior RAC review, with oversight/sanctioning of the local Institutional Biosafety Committee (IBC). Regarding study-related requirements for the polio vaccine, the IPHP states, "Most American adults have received a polio vaccine series. Inactivated polio vaccine is available for adults, but there is no recommendation for booster vaccination for employees participating in this research. Employees with uncertainty about their vaccination status or with any questions related to vaccination may contact EOHW [Employee Occupational Health and Wellness] for personalized advice." The investigators noted that wild-type polioviruses have been used for more than 100 years in laboratory research, including approximately 50 years in the prevaccine era without a single incident of accidental harmful exposure. PVSRIPO is a confirmed safer version of the polio type 1 Sabin vaccine, which is the live attenuated vaccine administered to more than 3 billion healthy infants worldwide in the past 55 years. Administration of PVSRIPO, even to an unvaccinated individual, would result in immunization.

The issue and concerns about potential exposures to PVSRIPO were addressed at length as part of a prior RAC review in 2004. The current procedures for use and unintended exposures are based on thorough RAC/IBC recommendations on the subject. The experience accumulated from more than 4 years of PVSRIPO use in the Duke clinic does not indicate a requirement for revising these procedures. Protective immunity to polio is universal. The investigators explained that relevant "exposure" to polioviruses requires active ingestion of the agent (e.g., health care professionals consuming the contents of syringes containing the study agent), which is an extremely remote possibility in a health care facility. Possible exposure (e.g., contact with remaining infusate in the CED apparatus upon catheter removal) is limited to smear contamination of gloves or similar events. Such events are mitigated by standard hygiene procedures, including disposal of gloves in medical waste containers and hand washing that are part of everyday routine in professional health care facilities. Experience indicates that even relevant exposure to PVSRIPO is not associated with any health risk to individuals.

The investigators clarified that the diameter (not the volume) of the recurrent tumor must be between 1 cm and 5.5 cm for a patient to be eligible for the proposed trial. The investigators noted that to date, the largest tumor treated was 2,163 mm^3; the patient is alive and doing well more than 13 months later. In addition, they clarified that only those "new or worsening neurologic deficits" that do not resolve fully, to pretreatment baseline status, will be considered a DLT.

Subjects given doses 1 and 2 in the dose-escalation trial never required steroids or other therapy, and they never experienced neurological decline/deficits after receiving PVSRIPO. They remain symptom free more than 4 years post-PVSRIPO.

Preliminary data suggest that disease response is not dose dependent. The investigators noted that this is a typical characteristic of oncolytic immunotherapy, because the immune response to virus infection is not generally governed by dose but rather by the quality of the stimulus. The key biological events shaping the immunologic response to intratumoral infusion of PVSRIPO include innate antiviral type 1 interferon (IFN) release from infected tumor cells, presentation of pathogen/danger-associated molecular patterns in destroyed tumor cells, infection of antigen-presenting cells (e.g., dendritic cells, tumor-associated macrophages, microglia), and a type 1 IFN response/pro-inflammatory stimulation in APCs; recruitment of immune infiltrates (e.g., neutrophils, macrophages, natural killer cells, CD4/8 T cells, B cells). These events occur over a wide dose range. Other factors to consider are that PVSRIPO is replication competent, and the initial dose to be delivered may be amplified upon intratumoral propagation. The most impressive clinical responses were observed in patients at the lower dose spectrum, which informs the dose de-escalation strategy.

The investigators provided an update on the outcomes for the 18 patients treated in the previous trial at dose level −1, 5×10^7 $TCID_{50}$. Nine of the 15 subjects with at least 2 months of follow-up had started Avastin, and two subjects died relatively early in the study. The investigators clarified that the two patients

who died rapidly received Avastin, but they passed away after they and/or their caregivers decided to discontinue all supportive therapy and pursue comfort care instead.

Contraindications to bevacizumab (e.g., intratumoral hemorrhage) and failure of steroids will be considered DLTs only if they meet the criteria for a DLT. Otherwise, as stated in the "special considerations" section of the protocol: "If there are adverse events or other circumstances prohibiting the use of bevacizumab, [the investigators] will use corticosteroids or surgery, or other interventions deemed more appropriate for the patient by the treating physician, if needed, to treat the inflammatory reaction secondary to PVSRIPO."

Radiographic analyses and progression data have been shown to be unreliable with immunotherapy in general. Thus, determination of progression of disease is not one of the objectives of the trial. A description of the imaging changes occurring after intratumoral inoculation of PVSRIPO is one of the exploratory objectives of the proposed trial, with the aim of trying to better understand the clinical effects of PVSRIPO and to evaluate the means to interpret such effects.

Experiments in xenotransplantation models and in syngeneic rodent tumor models, including nonimmunogenic models (e.g., CT2A glioma, B16 melanoma) clearly indicate therapeutic efficacy of a single administration. The team's ongoing adult Phase I clinical trial of PVSRIPO also suggests that a single intratumoral infusion can be effective, as recognized through the granting of Breakthrough Therapy Designation by the FDA on May 10, 2016. Preclinical studies have shown that multiple administrations of PVSRIPO may be helpful for therapeutic purposes, at least in the setting of very aggressive animal tumor models. Multiple dosing is an option, given that polioviruses, in contrast to many other oncolytic viruses, unfold significant replicative potential in the presence of pre-existing neutralizing antibodies. The investigators have contemplated multiple PVSRIPO infusions in brain tumor patients but point out that this approach is limited by the obvious burden of multiple intracranial surgeries in the glioblastoma multiforme (GBM) indication. They caution against making mechanistic parallels with other types of oncolytic viruses due to PVSRIPO's fundamentally distinct potential of engaging adaptive anti-tumor immunity.

To date, no wound healing complications have been observed in any patients following PVSRIPO infusion and use of the convection-enhanced delivery system. PVSRIPO infusion occurs through a catheter inserted with a stereotactic biopsy approach. The incision is about 10 mm long. The investigators commented that it is safe to initiate Avastin 7 days after stereotactic intracranial biopsy without concerns for wound healing.

The investigators addressed the reviewers' comments regarding the ICD and will revise the document accordingly.

2. Responses to RAC Discussion Questions

The major toxicity associated with the study intervention appears to be an intracerebral inflammation. The Preston Robert Tisch Brain Tumor Center at Duke University, which sees approximately 900 to 1,000 cases of newly diagnosed malignant glioblastomas per year, is by far the largest specialized neuro-oncology center in the country. A significant number of patients seen at Duke are enrolled in a clinical protocol, and specifically in immunotherapy trials. The team has rigorous procedures to evaluate, interpret, and report neurological toxicities. The SOPs for assessment and treatment of neurotoxicities were submitted with the protocol.

The incidence of inflammation/edema appears to correlate with dose. It is difficult to establish an unambiguous dose-effect relationship, however, because there are no quantitative tests to assess the extent of intracerebral edema/inflammation and because PVSRIPO is replication competent. Clinical and radiographic experience suggests heightened incidence of cerebral edema associated with treatment with the investigational product at doses exceeding dose level 2. The timing for when symptomatic cerebral edema occurs is patient dependent, but it ranges from 1 to 6 months after infusion of PVSRIPO and typically is seen approximately 2 months post-infusion, including in the dose level −1 subjects. To prevent the possible negative impact of corticosteroids on immunotherapy, Avastin is being used as first-line

intervention to control cerebral edema. The investigators report that mitigation of clinical symptoms attributed to cerebral edema has been observed with Avastin therapy and that the ease of controlling the edema with Avastin and evaluation of imaging patterns helps differentiate between cerebral edema associated with the study intervention and true disease progression. Corticosteroids are not part of the standard accepted treatment for cerebral edema and are only used for very short periods as a bridge while awaiting the initiation of Avastin (e.g., in cases where a patient becomes symptomatic of edema over the weekend).

Some patients with smaller tumor volume may have a lower need for steroids and Avastin than patients with larger tumors, due to greater available intracerebral space to accommodate the edema. Since clinical management now involves starting Avastin immediately upon evidence of cerebral edema, use of steroids is less of a concern.

The ICD will be revised to state that there may be no medical benefit from participating in this research study and to explain that the main goal of this study is to determine the most practical dose of study drug that can be given safely, not its effectiveness. Language regarding possible improvement in the patient's symptoms will be removed to avoid the impression that subjects might benefit directly. The consent states that the research participant's condition may worsen and that information learned from this study may benefit others in the future. The document explains that research studies are voluntary and include only people who choose to participate in them.

Dr. Gromeier provided additional details about the first group of participants (all adults) initially given the minus-1 dose of PVSRIPO. All participants had measurable recurrent disease. No resections were done under the protocol or in conjunction with virus delivery; the surgical resections were done prior to the study. At the time of catheter insertion, a biopsy is taken to confirm the presence of tumor. None of the participants in the minus-1 group who died received Avastin. Dr. Gromeier noted further that as is done in most neuro-oncology trials, the study team collects the brains of participants who have died on protocol, when possible, to gain as much information as possible. Consistent with this practice, the parents/families of participants enrolled in the pediatric study will be approached about donating post-mortem tissues (i.e., brains) for research studies. One participant in that initial group had a catastrophic explosion of the tumor, which expanded approximately 20-fold across both hemispheres within a 4-week period. This type of event is extreme and rare. There was no evidence that the investigational product was responsible for the event, and as Dr. Gromeier pointed out, no available therapy would have made a difference to this patient. Another patient initially who initially was doing well post-infusion developed an inflammatory response that progressively worsened despite intervention; his condition deteriorated, and his family withdrew all supportive care. Dr. Gromeier noted that this type of response is not uncommon and that the team takes extraordinary measure to make sure their patients do as well as they possibly can. The reality is that the successful immunotherapies, checkpoint inhibitors, and CAR T cells are associated with significant toxicity that is required to overcome immunosuppression in the cancer.

Dr. Gromeier explained that patients are not routinely biopsied for research purposes because the investigators believe this would be unethical. In a few instances, however, biopsies have been done when medically indicated. In one case, a patient's lesion disappeared following infusion of PVSRIPO, but about 6–7 months later, there was a new nodule next to the site of the treated lesion that also looked like a tumor. A biopsy indicated an extensive inflammatory response and multiple treatment-related effects from the chemotherapy, radiation, and Avastin that the patient had received in prior trials and as part of his cancer care regimens. He was re-treated with PVSRIPO but did not have a complete response. A biopsy of another patient revealed no signs of treatment-related adverse effects at the same time point as the patient with the new nodule. Dr. Gromeier pointed out that different levels of response are characteristic for immunotherapy and that it is unlikely that a homogeneous response would be found with biopsying.

The investigators noted that the FDA put some restrictions on the size of tumors that could be treated with the investigational product. The restrictions are the same for pediatric and adults patients and are specified as between 1 to 5.5 centimeters (cm) in diameter. The largest tumor treated to date was 2,163 mm^2; this patient has had a very positive response to the intervention and is doing well. The expectation that large amounts of interferon would be produced following infusion has not been observed. Based on

the small number of patients treated to date, there does not appear to be any relationship between the size of the tumor and either inflammatory toxicity or treatment response. Patient selection and the location of where PVSRIPO is delivered are very important factors, however.

Gadolinium is co-infused with the virus with FDA approval to predict the distribution of larger molecules in the brain. The main criticism regarding the use of gadolinium is that, because of its low molecular weight, it is not representative of large biological agents and therefore does not have good predictive value. Dr. Gromeier noted that gadolinium has been useful in addressing many of the problems with CED studies and delivery by improving infusion accuracy to ensure that the inoculum is within the area of the tumor. In contrast, MRIs alone are very inaccurate in reflecting the true extent of the tumor and guiding needle placement.

The investigators believe there is sufficient evidence to suggest further dose escalation. The greatest concern with a higher dose, however, is inflammatory toxicity. The data enclosed in the red boxes in the presentation table are for the four participants in the minus-1 dose group whose MRIs and clinical status are the similar to the first two participants in the dose-escalation phase who have done well. One additional participant given the minus-1 dose might also show improvement with the investigational product, but that subject's MRIs are different from others in the group. Participants have several consecutive MRIs in 2-month intervals to monitor the tumor mass; if the tumor shrinks over time, it is reasonable to assume that the subject is doing well. As the clinical research moved forward, the investigators have learned how to better gauge responses, given the persistence of these tumors and the challenges associated with immunotherapy. While two of the four improving participants in the minus-1 dose group received Avastin, the subjects who are the furthest along never required Avastin, which is the motivation to escalate the dose further. If the inflammatory toxicity response can be limited, higher proportions of patients might do well.

Dose-escalation/de-escalation studies have been conducted in animal GMB models, including in two established non-immunogenic, treatment-resistant rodent tumor models (i.e., CT2A glioma, B16 melanoma). Results of preclinical testing in these models indicate clear therapeutic efficacy of a single PVSRIPO administration. Efficacy is demonstrated from a low dose of 5×10^6, which is lower than dose level minus-2 in human adults, up to a very high dose of 5×10^8. Efficacy is lost as the dose tapers down to 5×10^5. The threshold for an effective dose above which there is no additional benefit has not been definitely determined yet but remains a goal of this research. Dr. Gromeier noted that de-escalation to dose level -1 (5×10^7) appears to be the closest to this threshold based on what is currently known. One notable difference between humans and the mouse models, however, is the lack of a clear inflammatory response in mice, underscoring the importance of clinical trials to discern the optimal effective dose for patients.

For the proposed trial, the starting dose will be 20 percent of the dose defined as optimal among adults with recurrent malignant glioma (i.e., the "minus 2" dose, 1×10^7). Dr. Gromeier clarified that although the proposed protocol is currently written as a dose-escalation trial, the investigators are not planning to increase the initial dose unless there is clinical evidence to support escalation. The investigators had written, and initially planned to proceed with, a Phase II study once the dose-escalation trials in adults were completed. However, the FDA advised the team to not submit the Phase II protocol and to continue to enroll patients in the Phase I trial. Per that recommendation, the proposed study was prepared, and an amendment to the IND for PVSRIPO to study the product in pediatric patients is pending FDA response/approval. Dr. Gromeier acknowledged that the study is not a classic Phase I design. He noted further that the protocol submitted for review by the RAC was written before the decision on dose de-escalation was made. The investigators plan to enroll six older children (age 12-18 years) first and then, if there are no safety concerns and with FDA approval, enroll six younger patients (age 5-12 years), in accordance with the FDA standard for pediatric trials. Only one dose will be tested, unless clinical evidence suggests otherwise. Dr. Gromeier noted that the size of the brain is the same in pediatric patients as in the adults and that pediatric GMBs are usually classified as in patients up to age 21. The disease is extremely rare in children younger than age 12. The investigators have identified no compelling rationale to have different doses in each subpopulation beyond what is planned. An amendment to the IND for PVSRIPO to study the product in pediatric patients is pending FDA response/approval. Dosing in

adults under the ongoing trial will be completed before dosing in children is started, pending approval of the proposed study.

The eligibility criteria for the trial are very restrictive and will streamline the recruitment and enrollment process. Dr. Gromeier noted that grade III and IV malignant glioma is very rare, with about 200 new cases reported each year. Given the inclusion/exclusion criteria and the incidence of this disease, the investigators do not expect to be overwhelmed with a large number of potential study candidates to screen.

Dr. Gromeier clarified that the statements regarding coverage of costs by the patient and/or insurance provider refers to standard-of-care aspects of the trial, such as the biopsy and overnight ICU stay, which usually are covered by insurance. He noted that it is not unusual to have problems with insurance providers; the language is somewhat vague but reflects reality. He agreed that patients should not bear these costs, especially when the subject is a minor; in these cases, the whole family (or at least several members of the family) travels with the child for the study visits. These issues have been discussed by the study team, which will be presented to the Duke IRB for final decisions as to what is covered.

Dr. Gromeier pointed out that the virus used in the investigational product is a significantly safer version than in the Sabin live-attenuated vaccine, which has been given to more 3 billion children worldwide over the past 50 years. He described the viral product as a safe version of a CDC-recommended pediatric vaccine. He noted further that the only way transmission can occur is through fecal-oral contamination. Thus, even in the worst-case scenario, there is essentially no risk of an adverse consequence to the exposed person. The Duke employee health office reviewed the health records of all persons who would plausibly be in contact with patients or patient materials (e.g., nurses, clinical care workers, pharmacists, study clinicians and other study staff) and confirmed that all have been immunized against polio. Because immunity is universal in the United States, and re-vaccination does not confer additional benefit or protection, re-vaccination is not recommended, even for lab workers and even with use of the wild-type neuro-virulent virus. Individuals who have not been vaccinated or who have severe acquired or inherited immunodeficiency syndromes (e.g., HIV) are rare but would be considered vulnerable and would require special precautionary measures. The study team is working with the health office to identify any such persons. Potential exposures will be identified and tracked as part of the monitoring plan. A detailed plan is also in place for the preparation and handling of the agent from the investigational pharmacy through delivery to a designated ICU nurse at the study site. Exposure would entail, for example, some smear contamination on a glove or as the catheter is being removed, which would be addressed by disposing of the article as contaminated medical equipment and biowaste. Spillage from the syringe would be a reportable event requiring a specific decontamination strategy using 10 percent household bleach. All SOPs for accidental exposures and precautionary measures are detailed in IPHP, which is distributed by the pharmacy to the nurses and the clinical trial staff; this plan is also appended to the clinical protocol.

Dr. Gromeier explained that the Duke University IRB will not review any materials until other approvals are in place. The team will work with the pediatric protocol writer at Duke to assure that the RAC's comments are taken into account and that a process for assent, including a separate assent form (or forms), is in place.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

1. Consider amending the protocol to update the current dosing plan to be consistent with current plans.

2. Strongly suggest amending the protocol, if appropriate, to be a dose de-escalation study even if there are no DLTs.
3. Suggested modifications to the consent form include:
    a. Clarify that once have received study drug, it's impossible for participant to fully "withdraw" from the study;
    b. Ensure consistency throughout that this is a safety study, and any benefits are very unlikely.
    c. Provide a mechanism for gaining assent from children through a separate form.
    d. Change term "doctor" to "investigator," and ensure the tone of the form is consistent with that meaning.
    e. Clarify this is a genetically modified virus and an experiment, versus a therapy.
    f. Clarify that this study has been performed in adults.
    g. Clarify the options of alternatives (hospice care, etc.)
    h. Consider the addition of a picture describing the treatment.
4. Consider developing a rational system for choosing participants.
5. Consider reviewing the policy for housing and reimbursement of participants and families.
6. Consider including payment for tests unique to this study.
7. Suggest that inclusion criteria should require that participants will be eligible for Avastin therapy.

G. Committee Motion 5

Dr. Curry summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Curry requested a vote, and the RAC approved these summarized recommendations by a vote of 10 in favor, 0 opposed, 0 abstentions, and 1 recusal (Dr. Whitley).

XII. Review and Discussion of Human Gene Transfer Protocol #1603-1509: Pediatric and Young Adult Leukemia Adoptive Therapy (PLAT)–03: A Pilot Feasibility and Safety Study of CD19t T-APC Vaccination Following CAR T Cell Immunotherapy for CD19+ Leukemia

Presenters:	Colleen Annesley, M.D., University of Washington Rebecca Gardner, M.D., University of Washington Michael Jensen, M.D., Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute
RAC Reviewers:	Drs. Donahue and Zoloth
Ad Hoc Reviewer:	Dean Lee, M.D., Ph.D., University of Texas MD Anderson Cancer Center

Dr. Whitley was recused from consideration of this protocol due to a conflict of interest. As a result of Dr. Whitley's recusal, Dr. Donahue chaired this session of the June 2016 RAC meeting.

A. Protocol Summary

ALL is the single most common type of pediatric cancer, and it is the leading oncologic contributor to childhood mortality. While current treatments with prolonged multidrug chemotherapy result in high overall cure rates in excess of 80 percent of patients, recurrent disease is a significant contributor to childhood cancer morbidity and mortality, with overall survival rates of only 40 percent to 50 percent. Any secondary or additional relapse has declining rates of survival with disease that is often chemoresistant. Salvage therapies that are effective and tolerable are urgently needed for these patients.

The proposed trial is an open-label, nonrandomized pilot study that will enroll pediatric and young adult research participants under age 18 with relapsed or refractory CD19+ ALL, with and without prior history of allogeneic stem cell transplant. Participants will have previously been treated or planned to be treated with CD19-specific CAR T cells on the second phase of Seattle Children's Hospital PLAT-2 Phase I protocol and subsequently met criteria to enroll on this study, PLAT-3. This pilot study seeks to examine the feasibility and safety of a different set of T cells that have a portion of the CD19 protein on their surface; these cells are called T-antigen presenting cells (T-APCs). Preclinical studies in immunodeficient mice and NHPs suggest that T-APCs can be infused without significant toxicities. However, the risks of T-APC infusions in humans are not fully known. Transient fevers and chills might be expected based on triggering residual CAR T cells to secrete cytokines. An additional risk for patients receiving donor T cells is development of GVHD.

In the proposed trial, the T-APCs will be derived from the patient's own peripheral blood mononuclear cells (PBMCs) that have been genetically modified using a lentiviral vector to express a truncated (i.e., cytoplasmic tail-deleted), human CD19-encoding transgene (CD19t). The patient population will be subdivided into three cohorts based on their CD19 antigen load (measured as in vivo CAR T-cell expansion) or loss of B cell aplasia. A flat dosing schedule of T-APCs is proposed based on the subject's weight. Children who weigh less than 25 kg will receive a dose of 2.5×10^8 cells, and those who weigh more than 25 kg will receive a dose of 5×10^8 cells. Patients will receive a full dose of T-APCs every 28 days for between one and six total doses. Up to 30 evaluable patients will be enrolled. Toxicity and feasibility will be monitored continuously for each patient, through 28 days after the last T-APC infusion. The design employs the Sequential Probability Ratio Test (SPRT), enabling early stopping for toxicity and feasibility.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. The protocol was found to warrant public review because it is the first-in-human trial to use this approach for enhancing CD19 CAR T cell expansion and proliferation, which is thought to be a key factor for clinical response to T-cell immunotherapy.

Two RAC members and one ad hoc expert provided written reviews of this proposed Phase I pilot trial. Dr. Zoloth submitted her written comments late and read them into the record during the meeting.

Dr. Lee's comments and questions focused mainly on how the study design handles two separate gene therapies and on the safety of the trial design. He inquired about the following issues:

- The rationale is needed to explain why irradiation of the T-APC is not used to provide additional safety. Is there a need for the T-APC to maintain replicative potential?
- The T-APCs should be recognized and eliminated in the process of stimulating CAR T-cell proliferation. If the T-APCs are ineffective at mediating a response from CAR T cells or are delivered after CAR-T have been rejected, would they persist in the patient much longer than anticipated, as described in the NHP experiments? How will this be assessed, and if present, will it be addressed?
- The submitted materials describe a 91 percent response rate with CD19 CAR T cells. The need for the T-APCs is in those patients who have early loss of CAR T-cell persistence, but the proportion of that subgroup of patients is not reported. What is the estimated proportion of patients receiving CD19 CAR T cells that would derive additional benefit from T-APCs (i.e., those who respond, experience loss of B cell aplasia (BCA), and have CD19+ relapse)? Specifically, the background information describes antigen loss at day 63 as predictive of CD19+ relapse, but only Cohort C directly uses early antigen loss as an enrollment criteria. Cohorts A and B use alternative surrogate markers (low antigen load, rapid decline on day 14), for which data is less supportive. The data provided are mostly a series of progressive associations without supportive statistics. More complete data on the predictive value of these surrogate markers as they relate to CD19+ relapse is therefore needed. In addition, because overlap between these criteria is not provided, it is not possible to estimate the proportion of all patients on PLAT-2 that are expected to meet at least one of the cohort criteria on PLAT-3. This information and an estimate of the

proportion of patients that are likely to receive T-APC gene therapy unnecessarily (i.e., would not have relapsed with CD19+ disease) should be provided.

- The protocol indicates in numerous places that patients will be removed from PLAT-2 if they enroll in PLAT-3. However, PLAT-3 has inadequate information about CAR T cells for a trial that infuses these cells. Issues related to the experimental infusion of CD19 CAR T cells (objectives, toxicities, vector maps, etc.) must be included in addition to information on T-APCs. If all subjects on PLAT-2 who have any of these three risk factors for CD19+ relapse are taken off PLAT-2 to be treated on PLAT-3, how does that bias the PLAT-2 statistics? Will those subjects be considered failures or will they be assessed as unevaluable and replaced?
- The predictive value of BCA duration with respect to relapse risk needs to be described more fully in the protocol. Dr. Lee provided an analysis based on the data presented in the protocol, which specifies that 33 subjects achieved remission, 17 of whom later relapsed. Six patients are indicated as relapsing with CD19-negative disease, which thus presumes that 11 patients relapsed with CD19+ disease. Because only 2 of 13 patients who maintained BCA at day 63 relapsed, at most it can be assumed that 9 relapsed with CD19+ disease among the 20 who lost BCA (these data are not included in the protocol). Further assuming that none of the 33 patients who achieved remission died of any cause other than relapse, then 2/13 (BCA) vs. 9/20 (lost BCA) yields a p-value of 0.13 (Fisher's exact). Thus, the primary premise on which this study is designed (i.e., that BCA is associated with CD19+ relapse) is not statistically significant. If these data are nonetheless true, and the surrogate markers for Cohorts A and B are 100 percent predictive, the intervention is 100 percent effective at restoring BCA, and BCA is 100 percent effective at preventing CD19+ relapse, then 11 patients will be treated with gene therapy unnecessarily for every 9 who are aided. Any reduction in predictive value or efficacy thus reduces the benefit ratio.
- If re-infusion has not been successful, and CAR T-cell loss is immune-mediated, are T-APCs anticipated to overcome the mechanisms of rejection?
- Approximately 25 percent of patients have low antigen load (<15 percent CD19+ cells); these patients, in turn, have shorter duration of CAR T-cell persistence. Was this difference statistically significant? Is this predictive of CD19+ relapse? What proportion of these patients had CD19+ relapse compared to patients with more than 15 percent CD19+ cells (i.e., positive predictive value of this criteria for needing T-APC)?
- Patients who have a ratio of day 10/day 14 CAR T cells that is greater than 1.5 are less likely to maintain persistence. How much less likely? What are the data and p-values to support this conclusion? Are these patients more likely to lose BCA? Are they more likely to have CD19+ relapse?
- What is the timeframe for enrollment in Cohort B? How soon after establishing day 14 loss of CAR T cells will patients be enrolled? Are they still eligible to enroll 3 months later? If patients receive T-APC infusions on Cohort B and then have loss of BCA, why would they be moved to Cohort C? Hasn't this already established failure of T-APCs?
- What is the evidence for choosing the T-APC doses that will be used in the proposed pilot study?
- The rationale for use of ongoing BCA as an inclusion criteria for receiving T-APC is needed, as this plan seems contradictory to the intent to use of T-APCs in Cohort A and loss of BCA as an inclusion criteria in Cohort C (except Cohort B, which requires ongoing BCA).
- Are there any criteria for toxicity in the study related to the CAR T cells? What if a subject cannot receive T-APC; will they be replaced?
- Most of Section 5 of the protocol applies only to CAR T cells, not to T-APCs. Specifically, if the lymphoproliferative disorder (LPD) arises from the infused T-APCs, there is no truncated epidermal growth factor receptor (EGFRt) through which cetuximab can work. This section and the interventions that would be given specific to T-APCs need to be clarified.
- In the drug information section of the protocol, there is no information given for CD19 CAR T cells, although these cells are clearly being given in this study.
- For patients who are taken off of PLAT-2 prior to receiving CAR T cells and then receive CAR T cells on PLAT-3 and experience life-threatening toxicities or death before receiving T-APC, it appears that their event will not be captured on either study. They have been taken off of PLAT-2 and are considered non-evaluable and replaced on PLAT-3.

- The assumptions of toxicity and feasibility need to be stated in the text (i.e., specify for P0 and P1 whether the assumptions signify probability of toxicity or of non-feasibility). Based on some information presented in the protocol, one might presume P1 is for feasibility.

Dr. Lee had the following additional questions and comments regarding Appendix M:

- Patients in Cohorts A and C will receive CAR T cells on this study. No vectors for expressing CAR are described, however.
- Two sections (M-II-A-1-d and M-II-A-2-b) refer to alternatives to CD19 CAR T cell therapy, instead of alternatives to CD19 T-APC. In another section, the alternatives listed are not alternates to T-APC, which is the therapy being assessed in this study. The alternative with respect to this study is to receive CAR T cells without receiving T-APC.
- Figure 2 is not a restriction map.
- Should the T-APC product be described as composed of "CD4 and CD8," instead of "CD and/or CD8"?
- Transfection efficiency and robust proliferation appear reasonable based on the data presented. What proportion of T-APC express the transgene after expansion? Is the 1:1 CD4:CD8 ratio maintained, and will the CD4:CD8 ratio of the final product (after expansion) be re-assessed?
- Clarification is needed as to whether there is an attempt to administer a product of defined composition? CD4 and CD8 are admixed at 1:1 prior to expansion, but there is no post-expansion validation or manipulation of composition to meet CD4:CD8 ratio or transaction levels, nor release criteria for expression of the CD19t transgene.
- It is not clear if an assessment for persistence of the T-APC (CD19t-expressing cells) is done only when a subject dies.
- There is no EGFRt indicated in several of the maps.
- The genetically modified cells being tested in this study are T-APC. There is no evidence that these genetically-modified T cells have ever helped patients with ALL.

Dr. Donahue had the following questions and comments:

- The biological therapy is called a vaccine, but it does not meet the NIH guideline definition of vaccine generally used for biological therapies (administration of a nucleic acid encoding a microbial immunogen); rather, it is an infusion of genetically modified cells. This should be clarified to avoid confusion.
- The investigators hypothesize that antigen stimulation will increase CAR T cell persistence. This hypothesis is supported with animal data showing successful T cell re-stimulation with MP1 in mice and ROR1 in macaques. In the macaques, the activated CAR T cells wiped out the APCs within 7 days of infusion. Dr. Donahue asked if there are any data showing similar stimulatory effects with the CD19 fusion protein, and if there are any data to support the selected dose of APCs. In addition, he asked whether a dose-ranging study would be more valuable than a single-dose study at this point in development. Any data to support the 28-day frequency of re-administration should be provided (i.e., is more frequent stimulation needed or is less frequent stimulation potentially effective). Overall the submitted preclinical efficacy and safety data are sparse. Additional information should be provided on how extensive the safety analyses in the animal studies were.
- In the animal studies, the APCs were administered 5 days after CAR T cell infusion, but in the proposed clinical trial, the APCs will be administered 21–35 days after CAR T cell infusion in Cohort A and at later time points in Cohorts B and C. Are there any data supporting these later initial infusion time points?
- Cohort A makes sense in that low levels of antigen stimulation seem to be the primary problem and increasing antigen stimulation may work to improve T-cell persistence. Dr. Donahue inquired about data showing that inadequate antigen stimulation is the underlying driver for loss of T-cell persistence in the patients in Cohorts B and C and whether there are any other factors that could cause loss of T-cell persistence in those groups. If there are potentially confounding factors for Cohorts B and C, would it be better to complete study Cohort A to obtain proof to support the study hypothesis prior to moving on to the other two cohorts?

- Given the potential for CRS in each cohort, the investigators should consider more robust assessment for normal organ function, including an estimate of glomerular filtration rate (GFR) for renal function and a requirement for normal (not just adequate) pulmonary and cardiac function.
- The plan for treatment of toxicities should include the parameters to define transient vs. persistent hypotension. The investigators should consider liberalizing the ability to use vasopressors, which under the current protocol are not considered until more than 40 minutes after the onset of therapy for significant hypotension.
- In the information in Appendix M on product release testing for CD19 APCs, clarification is needed as to whether the investigators plan to confirm transgene expression in the cells and look for persistent lentivirus in the final product.

Dr. Donahue commented on the following in the ICD:

- The cut-off age when assent will be sought and when younger children will be asked about their wishes, feelings, and understanding of the study needs to be specified.
- The statement, "You can quit the study at any time," needs to be amended to explain that there is no removal of the vaccine or the CAR T cells once administered.
- The information about results with CAR T cells to date is not accurate. Overall efficacy and toxicity data should be listed instead of suggesting "uniform" efficacy and safety for the therapy.
- The consent gives the impression that the "test dose" is the experiment. The whole study is an experiment. The word "therapy" should be modified to "potential therapy." The phrasing should be clarified to indicate that all doses are experimental.
- The clinical protocol suggests that the lymphodepletion chemotherapy step is usual clinical care. It is not clear, however, if all aspects of this step (e.g., dose and type of chemotherapy) are typical for the patient population, or if this step has been modified to fit the study requirements. If the latter is the case, that information needs to be clarified. Likewise, information about the timing and frequency of bone marrow testing and lumbar punctures (LPs) needs to indicate whether these are typical for usual care patients or if modifications have been made to the experimental protocol.
- The 15-year follow-up period is an FDA requirement, but the consent should explain that this is a regulatory issue and not an expectation of efficacy or survival for this disease.
- The average risk of ejection from the study for chemotherapy-related toxicities should be stated, particularly if additional or magnified doses of chemotherapy are used to prep for the study.
- The charts should be reformatted so that rows are not split between pages.
- The discussion on cetuximab needs to clarify conditions for use of this medication and implications for elimination of the CAR T cells.
- The intellectual property discussion needs to be separated from the other "gene therapy issues" and titled more accurately.
- Similar issues need to be addressed in the Cohort B consent form.

C. RAC Discussion

Dr. Zoloth had the following comments and questions for the investigators, as presented during the meeting:

- The proposed study is carefully designed with a strong rationale to investigate the safety of an innovative experimental product in pediatric patients with a grim prognosis. The protocol is complex and reflects a considerable amount of careful work and dedication to this patient population.
- Dr. Zoloth had two main ethical considerations, one involving the study design and one about the informed consent form. In studies of this type, inclusion is very important, and enrollment will be on a first-come/first-serve based on the patient's individual situation. Further information was requested as to how the investigators will ensure that patients have equal access to this study.
- In addition to the concerns about any Phase I clinical trial in children where the usual standard of risk and benefit is not applicable, this population may be particularly desperate and willing to enroll in any trial that suggests a possibility of any benefit because the patients have already tried

and failed at a long series of medical interventions. Given the high level of vulnerability of the target population, the consent process and the information in the consent are very important.

- As currently written, the protocol will admit children with a Karnofsky score as low as 50 percent, which indicates a very frail person. The investigators may want to consider a higher Karnofsky score given the rigors of participation. The consent process is also critical in such cases to ensure that patients and their families clearly understand what the study entails.
- The section on supportive care states that such care will be provided at the discretion of the investigator, as indicated by treating physician and the patient's medical team. It is not clear why supportive care is restricted and not given to all subjects. This section should be revised to reflect the information provided during the meeting.
- A subject may be discontinued from the study treatment at any time if the subject, the investigator, or the sponsor feels it is not in the subject's best interest to continue. Some of the possible reasons for study treatment discontinuation, particularly those that could be related to socio-economic status, and the role of the sponsor are problematic. Reasons behind certain criteria should be investigated; for example, if a minor misses appointments because his or her parents cannot get the child to the study site on time or as needed, it seems unnecessary to punish the child. While a role for the sponsor in deciding whether to close the study, further clarification is also needed to explain why the sponsor is involved in assessments for individual subjects, and the reasons the sponsor would have to make such a determination.
- The rating and classification of adverse events as serious or non-serious is confusing and inconsistent. The definition of a serious adverse event is very broad in one section of the protocol and includes events such as bronchospasm requiring intensive treatment in the emergency room or at home and blood dyscrasias or convulsions that do not result in inpatient hospitalization. The document later says that non-serious events include events where "hospitalizations occur on an outpatient basis and do not result in overnight hospitalization," which appears to imply that going to the emergency room with seizures or bronchospasms would be defined as a "non-serious" adverse event. Many of the complications and events that are defined as non-serious would be understood or considered serious if they occur in a child who has received an experimental therapy and who is already in poor health. The investigators should consider describing such events as serious in the consent form so that the subjects and their family have that understanding.
- The consents are very long (25 pages) and very detailed, which could present a challenge to potential participants as to how much of the document they will actually read. Dr. Zoloth found this aspect of the consents to be particularly problematic for a teenager or a parent whose child has failed conventional therapy and whose driving motivation is to get the child into treatment. Some parts of the consents are confusing. For example, the purpose of the charts in the documents is not clear; it would be preferable to include this information in narrative form. Dr. Zoloth suggested that the investigators develop two forms, one for children, who will be giving assent, and one for the parents or guardians who will give surrogate consent. Additional issues regarding the consent were raised for consideration:
    - The use of "you" versus "your child" needs to be clarified and made consistent, depending on the target audience for the document (i.e., who will be reading the consent).
    - The primary objectives of this Phase I trial are to assess the feasibility and safety of administering the investigational product, not to determine efficacy. The phrases "we hope to provide you with some benefit" and "what benefit will I receive from this trial" could easily be interpreted that there is some benefit from participating in the study, even if there are other qualifying statements or explanations.
    - The meaning of the phrase "deemed eligible" is unclear. If the investigators mean to say that they have decided that the patient is strong enough to take on the large risk and the entire study process, that is what they should convey.
    - Additional details about the process of lymphodepletion are needed. Saying that this procedure is done to "make room for the CAR T cells" does not describe what the patient will experience for this part of the study.
    - The risk of severe toxicities and CRS in particular is significant. These risks were clearly described in the presentation but not as well in the consent documents. It is important to be

as clear and transparent as possible so that subjects have a good understanding of the study risks.
    - The reference to “decreased ability of [the] heart to pump” as a possible side effect of severe CRS and life-threatening hypotension could be interpreted that a cardiac arrest requiring resuscitation could occur.
    - The terms “doctor/patient” should be changed to “investigator/subject” throughout the consent document to more accurately describe the nature of the roles of and the relationships between the participants and the study investigators.
    - The consent should also say that the modified cells cannot be removed once they are administered and that “withdrawal” is withdrawal from monitoring and evaluations that may be important for safety assessments. The statement that subjects could be taken off the protocol if they cannot keep enough of the study appointments mirrors the comments above that the reasons why a subject has missed visits should be pursued, and that efforts should be made to retain subjects, and children in particular, regardless of their socioeconomic condition or their parental or guardian situation (if those are factors for not following the visit schedule). Dr. Zoloth pointed out that consent is not just a one-way agreement or process. The subjects agree to undergo invasive procedures and testing so that the investigators can explore an important hypothesis. The investigators, in turn, are morally bound by the informed consent document. A Phase I clinical trial in children is a tragic enterprise, but the investigators have an ongoing obligation to do the best by these children as possible, which requires a very high standard of proof.
    - The entire section on birth control and another section about nursing seems needlessly optimistic. Dr. Zoloth acknowledged that this information is standard, but she considers it to be inappropriate for these very ill subjects with a very grim prognosis who are facing a Phase I clinical trial. It gives the impression that the investigational product will work and that the subjects will be cured and go on to have and then nurse healthy babies.
    - The “alternative treatments” section of the document needs to clarify whether any alternatives are available; if so, the consent should specify what they are and whether they include medical and/or non-medical options.
    - The section on privacy and confidentiality needs to explain that because the subjects’ cells are used, their genetic information is at stake. Provisions will be in place to protect privacy and confidentiality, but there is no actual anonymity.
- Dr. Zoloth again pointed to the trend across clinical trials to cover fewer and fewer aspects of a trial, even when these components are required to participate. She commented that these are moral and ethical decisions made by the investigators, sponsors, and study institutions. Requiring participants to cover the cost of research-related injuries and “standard care” procedures and tests adds to patients’ burden by their having to take on the additional financial risk of these costs. Dr. Zoloth noted that no insurance company will pay for research-related injuries incurred in Phase I clinical trials. The statements in the consents about coverage of expenses by the insurance provider should be removed. The team should focus on setting aside some funds for this purpose.

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- Drs. Donahue and Lee went through their comments and the investigators’ responses to their queries and suggestions. They found the presentation to be clear and their concerns and questions submitted with their written comments to be well addressed.
- Dr. Donahue noted the complexity of the proposed experiment and the multiple variables in play, including the dose of T-APCs, the dose of CAR T cells, and the timing of dosing, all of which can affect both toxicity and potential efficacy. The investigators acknowledged that the number of variables that can be evaluated is limited and will focus on the doses of the study agents and the frequency and timing of re-administration. Dr. Donahue pointed out that given the large body of data that will accumulate, some signals might be obscured because of the timing of dosing or the dose itself. For example, it may be difficult to discern whether dosing on a 14-day basis would be better than the planned 28-day basis, or if fewer side effects would be seen at a half-log lower dose.

- Dr. Donahue requested additional information on the number of variables that were evaluated as part of the safety analysis in the animal studies, including how extensive or detailed the observations were and whether the team used a set of prospective criteria for this assessment.
- Dr. Donahue had some concerns about pretreatment testing to determine "adequate" vs. "normal" organ function and the related inclusion criteria for this study. Most patients will not have a lot of reserve at the time of a cytokine release event. Thus, if they already have, for example, even minimally impaired cardiac, pulmonary, and/or renal function, these problems will be further exacerbated under the stress of CRS and other adverse events. To minimize these risks, organ function for all patients should be within solidly normal ranges, not slightly above or below normal, as an added safeguard, so that they have as much organ reserve as possible when toxicities or other stressors occur. Given these concerns, Dr. Donahue recommended that the investigators consider revising the eligibility criteria to remove these potential confounders. However, Dr. Lee questioned whether it would be appropriate to change the enrollment criteria for the proposed trial so that they differ from the criteria for PLAT-2. The impact of having to meet different criteria to receive the CAR T cells simply because they have been moved to a different protocol needs to be weighed carefully. He suggested keeping the criteria for this study as close to those for PLAT-2 as possible, as currently planned.
- Dr. Lee commented that based on what is known so far about infusing genetically-modified T cells and T cells themselves, the proposed trial is likely to be very safe with respect to infusion of the T-APCs and should involve no more risk than the toxicities associated with CAR T cells, which also have the potential for benefit in some patients. He noted further that this patient population is a high-risk group for which effective and tolerable therapies are needed.
- Dr. Lee continued by identifying three major areas of concern: Confusion and/or ambiguity as to what the real gene therapy is in this study, inclusion criteria that are very broad and may overlook a specific group that needs therapy, and the impact of PLAT-2 and PLAT-3 on each other particularly in relation to patient selection and enrollment and statistical modeling.
    - The primary scientific aims and study objectives of this research, as specified in the protocol, focus on this being a therapy study of T-APCs, but the study actually includes two modified gene products, CAR T cells and T-APCs. This point appears to be missing across all documents, including Appendix M, the consents, and the protocol. It needs to be stated explicitly and consistently that this protocol involves two gene therapies with the exception of arm B, which involves infusion of only the T-APCs. Information about the specific gene modifications, toxicities/risks, and any potential benefits needs to be addressed independently for each of the two gene therapies so that it is clear that two products are being administered and studied. It might have been simpler to have a second study in which subjects were enrolled or co-enrolled after being on PLAT-2, but there likely would be challenges with that approach as well.
    - The section on inclusion criteria for each of the study arms create broad catchment groups for this patient population. Dr. Lee commented that the group that should be targeted to treat with this therapy includes patients that ultimately have a CD19-positive relapse. To date, three biomarkers of CD19-positive relapse that occur in succession over the period of therapy have been identified: rapid decline in CAR T cells post-dosing, loss of B cells and whether or not the patient has continued B-cell aplasia, and low disease burden. The investigators clarified in the written responses that loss of B cell aplasia indicates that the CAR T cells are already lost and that, as a result, there is no reason to try the T-APCs. A rapid decline in CAR T within 10–14 days of administration predicts loss of B cells, which, in turn, predicts relapse of CD19-positive disease. Low disease burden is associated with lack of stimulation, which predicts rapid loss of CAR T cells and, in turn, B-cell aplasia to again predict CD19-positive relapse. Dr. Lee noted that the numbers for how those successively increasing circles overlap is not readily apparent in the protocol. The predictive value of these markers is evident from the responses of the 42 patients enrolled in PLAT-2. Of these subjects, there were 14 that would have been eligible for Cohort A because they had low disease burden. Of the 28 that would then proceed, another 11 subjects could be pulled out for Cohort B because of rapid loss of CAR T cells. It is not clear how many of the 17 remaining subjects would eventually have lost B cell aplasia. Further consideration of these cohorts indicates that at least 9 of the 11 patients who went to Cohort B would have relapsed

CD19-positive disease. Thus, by pulling out 25 patients for treatment, only 9 would be expected to respond, while 16 would have been unnecessarily administered the investigational product(s). Some CD19-positive disease relapse and loss of B cell aplasia would be due to immune rejection of the T cells, not because of loss of adequate APC stimulation; thus, even if a therapy is 100 percent effective, for a proportion of patients, it would not be the right intervention.

- Regarding the relationship between PLAT-2 and PLAT-3, Dr. Lee noted that the protocol indicates in numerous places that patients will be removed from PLAT-2 if they enroll in PLAT-3. The question of how PLAT-2 manages data reporting of toxicities related to CAR T cells that subsequently occur on PLAT-3 is important and needs to be addressed through a plan that specifies how reporting of toxicities across gene therapy trials will be handled.

- Dr. Atkins followed up Dr. Lee's comments on the relationship between PLAT-2 and PLAT-3 and subject selection for enrollment into one of the three cohorts in PLAT-3. He noted that it isn't an absolute that a subject with an initial low tumor burden, rapid drop in CAR T cells, or loss of B cell aplasia will relapse. Thus, some patients on PLAT-2 who would fall into any of the PLAT-3 cohorts will remain relapse free. Taking this possibility into account, the investigators were asked whether the treatment approach in the proposed trial could harm patients who might already have been effectively treated by the CAR T cell approach in PLAT-2, and is this issue raised in the protocol or consent form (i.e., if patients were effectively treated in PLAT-2, the intervention in PLAT-3 could negatively impact that endpoint). Dr. Cho requested additional information about the implications for interpretation of results from PLAT-02 with removal of subjects from that study and their inclusion in PLAT-3.
- Dr. Atkins also inquired about splice variants in CD19 relapses and whether patients have CD19 relapses because they have lost their CAR T cells or because the CD19 is expressed differently so that the CAR T cells no longer recognize it.

D. Investigator Response

1. Written Responses to RAC Reviews

The following factors were taken into consideration with respect to development of the CD19t T-APC product that pertains to consideration of product irradiation:

- The infusion of viable non-irradiated T-APCs was found to be safe and biologically active with respect to inducing the proliferation of engrafted CAR T cells in the NSG mouse and two independent NHP models.
- The CD19t T-APC product functions by presenting the native CD19 extracellular domain on a cellular plasma membrane. The capacity of T-APCs to synthesize the CD19t transgene, present cell surface CD19t, and remain viable and competent for APC activity for the durations of time necessary to circulate and encounter a CAR T cell is expected to be compromised by lethal irradiation. Apoptotic, irradiated T-APCs are expected to be rapidly cleared, particularly during first pass transit through the pulmonary vasculature.
- CD19t T-APCs are biologically minimally capable of inducing toxicity themselves. CD19t without a cytoplasmic tail is devoid of CD19 signaling activity.
- The biosafety of the third-generation self-inactivating (SIN) lentivirus vector has a robust record regarding RCL negativity and cellular transformation in human T cells. Because the treated patients have been previously exposed to lentivirally transduced CAR T cells, risk of T-APC exposure is incremental.
- The trial is designed to infuse CD19t T-APCs to patients with CAR T-cell functional persistence as quantified by ongoing B cell aplasia wherein elimination of CD19t T-APCs is anticipated.
- The prognosis of enrolled patients with refractory ALL is dismal, and the subsets of patients to be enrolled in PLAT-3 are those with the worst prognosis for sustained remission after a CD19 CAR T cell–induced remission. Given the status of these patients, the investigators consider the risks and benefits of this Phase I trial to be appropriately balanced.

The trial is designed to selectively administer CD19t T-APCs to research participants with functionally persistent CD19 CAR T cells. Thus, the expectation is that T-APCs will be cleared. The trial includes monitoring for T-APC persistence by CD3xCD19 flow cytometric assessment of PBMCs and bone marrow at multiple time points. The investigators do not expect the persistence of these CD19t-expressing T cells to cause toxicities should they not be cleared. The CD19t, truncated human CD19 transgene, is not biologically active and serves as a transduction marker. Potential methods to specifically ablate them could be pursued using anti-CD19 mAbs, anti-CD19xCD3, or reinfusion of additional doses of CD19 CAR T cells.

The investigators provided a detailed reporting of outcomes to date (as of May 17, 2016) in the Phase I arm of PLAT-2, which has a 93 percent complete remission (CR) rate (39/42 subjects) and a 1-year relapse-free survival rate of 53.3 percent. Of the 17 relapses that have occurred thus far, 11 have been CD19+. Due to the sample size of the Phase I arm of PLAT-2, it is not currently statistically powered to make determinations of effects of duration of persistence on the durability of remission in a definitive manner. In addition, the Phase I arm of PLAT-2 was not powered to identify predictors of relapse. However, strong trends indicating that the duration of functional persistence of CD19 CAR T cells, as measured by the duration of B cell aplasia (a risk factor for subsequent relapse of CD19+ leukemia after remission), have been observed. Of the 11 CD19+ relapses, 2 subjects had functional T-cell persistence at greater than 2.1 months, whereas the other 9 relapsed subjects had functional T-cell persistence at less than 2.1 months. Of the subjects with persistence greater than 2.1 months, 47 percent relapsed with CD19+ disease, whereas for those with persistence greater than 2.1 months, only 17 percent relapsed with CD19+ disease ($p = 0.13$). While not statistically significant, this represents a strong trend in a Phase I trial with small numbers of patients treated. Only three patients with short persistence remain in remission without having proceeded to consolidative transplant, two of which have not yet reached 6 months of follow-up and are in work-up for transplant. The two subjects with CD19+ relapse with persistence greater than 2.1 months had persistence of 3.07 and 3.93 months, respectively. Of the 10 subjects with persistence of 4 months or longer, there have been no CD19+ relapses. Of note, these data account for only 29 subjects. The analysis excluded three subjects who proceeded to transplant prior to 2 months and had ongoing CAR T-cell persistence, three subjects who were non-responders, and three subjects who had CD19-negative relapse prior to 2.1 months. The remaining four subjects had not reached 2 months of follow-up at the time of the data cut-off.

The central hypothesis of the proposed PLAT-3 trial is that dosing patients at risk for short durations with functional CAR T-cell persistence with CD19t T-APCs will enhance persistence and decrease the risk of subsequent relapse with CD19+ ALL. The investigators analyzed the PLAT-2 Phase I data to assess patient features associated with relapse. This assessment identified the following cohorts and the rationale for how the cohorts were defined:

- Cohort A: These patients had a low CD19 antigen burden at the time of lymphodepletion, that is, less than 15 percent CD19+ leukemic/B cell burden in the bone marrow. From the Phase I PLAT-2 patient cohort, 14/42 (or 33 percent) of subjects fell into this category.
- Cohort B: PLAT-2 Phase I arm patients with greater than 15 percent CD19+ leukemic/B cell burden in the bone marrow have a significantly longer median duration of persistence (4.1 months vs. 1.7 months, $p = 0.008$) with a hazard ratio of 0.32. The investigators acknowledged that it is difficult to determine exactly how long CAR T cells need to persist in order to be protective against a CD19+ relapse; however, a multivariate analysis of a time-to-event endpoint (i.e., time to loss of BCA), pre-lymphodepletion CD19 antigen burden (<15 percent vs. >15 percent) is the only factor significantly associated with duration of BCA ($p = 0.04$). The multivariate model included the following baseline variables: dose level, conditioning regimen, age, gender, and CD19 load at time of apheresis.

  Among the subjects with high antigen burden (>15 percent), 11/42 (or 26 percent) of all subjects on PLAT-2 would qualify for Cohort B. Among subjects with high antigen burden, however, there are subjects who do not have long-term persistence of the CAR T cells, and additional markers are required to predict which patients will have short durations of BCA and higher relapse risk. In this group of patients, a ratio greater or equal to 1.5 of the percentage of CAR T cells in the blood

(on day 10 vs. day 14) demonstrates a trend towards rapid loss of CAR T-cell persistence prior to 2.1 months.

- Cohort C: This cohort will enroll patients with less than 6 months of BCA who will undergo a second round of lymphodepleting chemotherapy, a second dose of CAR T cells, followed by T-APCs. This cohort will provide safety and efficacy data for redosing after an initial response to CAR T cells. It is possible that a subject would decline participation in Cohorts A and B but may opt to participate after loss of CAR T cells; the number of subjects for this cohort is therefore difficult to estimate. The investigators do not expect all subjects enrolled in PLAT-2 and eligible for PLAT-3 will participate in the proposed trial. It is likely that some subjects will decline participation, particularly because the second phase of PLAT-2 will enroll at several sites. Further, patients may choose not to enroll in PLAT-3 if they are not in the Seattle area.

The investigators explained that prior to implementation of the PLAT-3, current versions of the protocol and consent documents will be revised to add detailed information about the CAR T cell product in addition to the information regarding the T-APC product based on RAC, IBC, IRB, and FDA inputs. Subjects enrolled on PLAT-3 Cohort A will be replaced in PLAT-2 because they will be removed prior to receiving an investigational CAR T-cell infusion under PLAT-2. Subjects who go on to Cohort B will be considered based on an analysis of duration of BCA per the PLAT-2 statistical analysis plan. These research subjects will be included in the overall efficacy analysis for response rates if they have a response evaluation done prior to T-APC dosing. Subjects in Cohort C will be fully evaluable on PLAT-2 as they will have already lost persistence of CAR T cells.

The investigators acknowledged that not every research subject in PLAT-2 who is eligible for PLAT-3 will have a short duration of CAR T-cell persistence and thus potentially benefit from T-APC dosing. The only cohort in which 100 percent of subjects will have had early loss of CAR T cells is Cohort C. The investigators hypothesize, however, that the ideal strategy is to promote persistence of the initial infusion of CAR T cells, since not all treated patients with CD19 CAR T cells are able to tolerate another round of lymphodepleting chemotherapy, with risks of CRS, and neurotoxicity. The research subjects who are eligible to enroll in PLAT-2 and potentially into PLAT-3 will have an overall short-term mortality rate of more than 80 percent. Although PLAT-2 has an encouraging 1-year relapse-free survival rate of approximately 50 percent in this patient population, there clearly is room for improvement. A 50 percent risk of a lethal relapse warrants additional investigative options, such as those proposed in the PLAT-3 protocol.

T-APCs are not expected to overcome the mechanism of graft rejection. The trial is designed to infuse patients with ongoing persistence of CD19 CAR T cells, as assessed by BCA or by the re-engraftment of CAR T cells in which achieving a second BCA status occurs. In both of these settings, CD19t T-APCs are expected to be eliminated. Ongoing duration of CAR T cells appears to protect against CD19+ relapse. Therefore, the subgroups chosen for this study are predicted to have a greater chance of loss of CAR T-cell persistence. It is possible that these subgroup analyses will not reach statistical significance based on powering of a Phase I trial for safety as opposed to efficacy outcomes. The aim of this pilot study is to look at safety of T-APCs and their effect on the duration of CAR T-cell persistence.

Regarding the timeframe for enrollment in Cohort B, the intention is that subjects would be enrolled onto PLAT-3 within a week of the day 10/day 14 ratio determination. The investigators will clarify that the timeframe of enrollment from determination of the day 10/day 14 ratio to be within 2 weeks and that subjects must be enrolled by day 28 after CAR T-cell infusion on PLAT-2. Subjects enrolled in Cohort B who lose persistence of the CAR T cells (as determined by recovery from BCA) during the time of manufacturing the T-APC would instead be eligible for screening for Cohort C; these subjects would then receive additional lymphodepletion and a second infusion of CAR T cells, with the requirement of developing BCA prior to T-APC dosing. Subjects who have already received the T-APC infusion in Cohort B will not subsequently be moved over to Cohort C.

The rationale for the T-APC doses is three-fold. First, T-APCs have not demonstrated toxicity, as would be expected, because there is no activation mechanism intrinsic to CD19t. The bioactive entity capable of inducing toxicities (CRS/neurotoxicity) is the previously engrafted CAR T cells. Given that the numbers of

CAR T cells in patients will be variable, the investigators chose to fix the dosing of T-APCs to better study the relationship between the two products in this Phase I trial. If dose escalation of T-APCs were to be performed in the context of variable CAR T-cell engraftment, the risk of not obtaining a tolerability assessment is enhanced. Second, T-APC doses were extrapolated from NHP studies showing efficacy and safety. Although NHPs in these studies received 1×10^8 cells/kg, PLAT-3 will use a more conservative maximum dose of 2.0×10^7 cells/kg to 2.5×10^7 cells/kg. Third, PLAT-3 has rigorous early stopping rules for excess toxicities; should these toxicities occur, the study team will work with regulatory committees to adjust the dose for subsequent patients.

The absence of ongoing BCA indicates the loss of functional CAR T-cell persistence. Without ongoing persistence of CAR T cells, there is a lack of rationale for T-APC infusion because the patient has no cells capable of responding to T-APCs. The goal in Cohort A is to intervene with T-APC infusion prior to the loss of CAR T cells to promote their ongoing persistence and ultimately reduce the risk of CD19+ relapse. Cohort C subjects have lost BCA, so they do not have functional CAR T-cell persistence. These subjects will have an attempt at re-engraftment of CAR T cells and if successful, would subsequently be eligible for T-APC infusions.

The investigators will make information about toxicities attributable to CAR T cells alone consistent across the protocol. Such toxicities will be tracked prior to the first dose of T-APCs. After the first T-APC dose, the toxicity criteria apply either to T-APCs (e.g., during the infusion itself) or to a combination of the T-APC effect on CAR T cells. The protocol has specific stated criteria to proceed with the first dose of T-APCs (i.e., the test dose) that requires patients to have fully recovered from CRS/neurotoxicity after CAR T cells.

The protocol will be revised to specify that cetuximab will be given for CRS and LPD arising from the infused T-APCs and prolonged BCA and that EGFRt has the potential to be used as a suicide gene with cetuximab.

Research participants who receive CAR T cells on PLAT-3 will be assessed for toxicity related to the CAR T cells even if they do not undergo T-APC dosing. Subjects who are unable to receive T-APC due to toxicity from CAR T cells or because of a manufacturing issue are counted as a failure of feasibility and will be replaced for the safety analysis of T-APC dosing on PLAT-3.

The investigators provided additional information and details regarding subject selection for PLAT-2 and PLAT-3, subject replacement, and potential bias and impact on data analyses. The PLAT-3 design uses the SPRT, a well-established standard method for sequential decision-making with small samples. The design implements four SPRTs separately and simultaneously: one toxicity SPRT for each cohort, and one feasibility SPRT for the entire study population. The parameters for the three toxicity SPRTs are identical, and they are also very similar to the feasibility SPRT parameters. This similarity is an incidental side effect of the fact that the boundaries for acceptable and unacceptable rates for toxicity and feasibility are relatively similar for the proposed trial. However, at no point are toxicity and feasibility compared to each other in a hypothesis test. Another factor potentially contributing to the reviewers' and committee members' questions is that the feasibility SPRT chart submitted with the protocol had a wrong y-axis label, referring to toxicities. The investigators apologized for this error and provided and presented the correctly labeled chart. The charts include "red," "yellow," and "green" zones that will be used in determining whether to proceed with, pause, or stop a cohort or the study.

In each SPRT, two competing hypotheses about the rate of the same endpoint are repeatedly compared. The toxicity SPRTs contrast the hypotheses of less than or equal to 20 percent toxicity (good) and greater than or equal to 50 percent toxicity (bad). The feasibility SPRTs contrast the hypotheses of less than or equal to 20 percent feasibility (bad) and greater than or equal to 50 percent feasibility (good). The two additional parameters specified for each SPRT are Type I/II error rates controlling the aggressiveness of early stopping for each hypothesis. In both cases, these parameters were chosen to produce an overall more conservative design in terms of patient safety. After each evaluable patient, cumulative toxicities (for that patient's cohort) and feasibilities (across all cohorts) are tallied, and the two SPRT charts are consulted. If the additional data point lands the cohort/study in the "red zone" for either chart, it means

that a detrimental hypothesis had triumphed over a beneficial hypothesis, and the relevant part of the study will stop. If it lands in "green zones" of both charts, then the study team might have the option of asking for an early successful end to a cohort or to the entire study, if all four charts are in their "green zone." If the situation is a mix of "green" and "yellow," it means that there is insufficient evidence to stop the trial before its pre-allotted sample size, and the study and cohort continue. Feasibility will be defined as the inability to administer the first dose of T-APCs secondary either to a manufacturing issue of the cells or due to unacceptable toxicity after the CAR T-cell infusion (precluding infusion of the T-APC).

The investigators clarified that the only alternative to receiving T-APCs following CAR T-cell infusion is to not receive T-APCs, as is most applicable to Cohorts A and B. There are no other investigational agents or interventions available to therapeutically alter the persistence of CAR T cells following a CAR T-cell infusion. There are, however, alternatives to CAR T cells and alternative approaches if the CAR T cells fail, including participation in early-phase trials of novel chemotherapeutic agents. These alternatives are also relevant for Cohort C patients.

The T-APC product will be manufactured from patient apheresis-derived purified CD4 and CD8 T cells. The product will be an undefined mixture of CD4 and CD8 T cells based on available numbers of cells in the starting material. For some patients, all of the CD4 or CD8 T cells may be consumed in making the CD19 CAR product; these patients will have T-APCs that are composed of either CD4 or CD8 cells. The product will therefore be composed of either CD4 or CD8 cells, not CD4 and CD8 cells, depending on the patient.

Validation of data suggests that for most patients, 60–70 percent of the product's T cells will express CD19t. The dose to the patients will be based on CD19t+ cells. The product will not be formulated for fixed quantities of CD4 versus CD8 T cells.

T-APCs will not be a defined composition product in this trial other than they are CD3+ and express CD19t. T-APC persistence will be tracked by flow cytometry for CD3xCD19-expressing T cells.

The team adheres to FDA guidelines on product testing for vector. CD19t expression by flow cytometric assessment is a test that is used to formulate the specified number and dose of T-APCs for cryopreservation.

The CD19t lentivirus vector does not encode EGFRt, but an EGFRt construct is co-expressed with the CAR and serves as a tag (with no signaling capacity) and an enrichment marker. EGFRt binds to cetuximab and can be used as a marker of transduction. During culturing, the CAR-transduced cells with the EGFRt tag can be selected out and potentially used as a suicide mechanism with administration of cetuximab to patients to ablate their CAR T cells.

The consents and Appendix M will be revised to clarify that there is no evidence to date in humans to show that T-APCs have helped patients with ALL.

The investigators provided the following information regarding results of preclinical studies in response to the reviewers' comments. This information was also presented during the meeting.

In in vitro co-culture, CD19t T-APCs manufactured under GMP SOPs trigger CD19CAR T cells for cytolysis and activate cytokine secretion. Due to the observation that the anti-human CD19-specific tumor-targeting single-chain variable fragment (scFvs) FMC63 used in the clinical PLAT-2/3 trials does not cross-react with rhesus CD19, the investigators did not pursue NHP studies with this CAR or huCD19t T-APCs. However, the CD20-specific CAR reacts with rhesus CD20, and data on the rhesus monkey model show that following lymphodepletion, the adoptive transfer of autologous CD20 CAR T cells induces B cell aplasia, cytokine storm, and neurotoxicity. The initial CD20 T-APC work in this model has shown the following: (1) CD20 T-APC infusions are non-toxic in animals (similar to prior NHP studies by Berger et al.) that experienced CRS and neurotoxicity upon initial CAR T cell engraftment; (2) CD20 T-APC infusions after persistent CAR T cells have disappeared are ineffective, and (3) CD20 T-APC dosing prior to disappearance of CAR T cells induces their proliferation 10-fold (from 9 to 93 cells/μL in

peripheral blood) without CRS or neurotoxicity. These safety data represent an aggregate of four NHPs dosed to date without discernable toxic side effects. The T-APC cell dose described in the Berger NHP model that was both safe and effective for inducing in vivo expansion of CAR T cells was 100 million cells/kg. The investigators on the proposed trial have elected to use a more conservative dose of 20–25 million T-APCs/kg.

Regarding the query about conducting a dose-ranging study instead of a single-dose study, the investigators noted that T-APCs in and of themselves have not demonstrated toxicity, as would be expected, because there is no activation mechanism intrinsic to CD19t when expressed by a T cell. The bioactive entity capable of inducing toxicities (i.e., CRS/neurotoxicity) is the previously engrafted CD19 CAR T cells. Given that the numbers of CD19 CAR T cells present in patients will be variable, the team chose to fix the dose of T-APCs at 20–25 million T-APCs/kg to better study the relationship between the two products in this Phase I trial. If dose escalation of T-APCs were to be performed in the context of variable CAR T-cell engraftment, the risk of not obtaining a tolerability assessment is enhanced.

The investigators agreed with the reviewers that dosing frequency will be an important parameter to fine tune. The initial 28-day dosing cycle is based on the expansion curves seen in the NHP models and reflects the T-APC manufacturing feasibility for creating multiple doses of T-APCs. Data generated from the proposed trial may provide compelling signals to modify dosing frequency. The investigators pointed out that any such modifications would be pursued only with full regulatory review and approval.

The timing of T-APC dosing will differ by cohort. For Cohort A, there will be a delay in the initiation of T-APC dosing until after the risk period for severe CRS and neurotoxicity resulting from initial CD19 CAR T cell engraftment has passed. Data from PLAT-2 Phase I suggests that risk of graft loss occurs in the first 21-35 days after CAR T-cell infusion, a timeframe in which most patients have recovered from CRS and neurotoxicity. For Cohorts B and C, the timing is different because of the clinical scenarios. For Cohort B, timing of T-APC dosing is predominately a manufacturing issue because subjects in this group will be enrolled after CAR T-cell infusion, when persistence tracking reveals a rapid decline in CAR T cell numbers. Once identified, the T-APC product will take approximately 21 days to manufacture and release for clinical use. Cohort C patients will undergo repeat lymphodepletion and CAR T-cell infusion while T-APCs are manufactured from cryopreserved T cells.

The investigators acknowledge that the mechanisms for CAR T cell loss in subjects identified for Cohorts B and C are likely to be variable. They hypothesize that in Cohort B patients, a rapid decline in CAR T cell frequencies following initial engraftment may be due to intrinsic properties of their autologous CAR T cell product that make CAR T cells more antigen-dependent for ongoing persistence. Alternately, some subjects will lose CAR T-cell engraftment because of anti-transgene rejection responses and would be eligible for Cohort C. These patients are not expected to re-engraft and would not qualify to receive T-APCs because they would not achieve the eligibility threshold of B cell aplasia. Given the possible heterogeneity in mechanisms, to find T-APC dosing to be safe, the study will proceed with all three cohorts because the activity in one cohort would not necessarily predict the safety and bioactivity in the other cohorts.

There is a minimal risk that persisting CD19 CAR T cells will not respond to subsequent T-APC dosing. The correlative studies planned for this trial, however, will define this response by quantifying both CAR T cell and CD19t T-APC frequency in peripheral blood and marrow in serially acquired patient specimens.

To date, the data from the NHP model have not demonstrated recrudescence of CRS or neurotoxicity following T-APC dosing. The eligibility criteria for infusion of T-APCs include normal creatinine for assessment of renal function; the protocol will be amended to include the additional requirement of a normal GFR per patient age. In addition, an oxygen saturation of 90 percent or higher on room air will be required prior to all subsequent doses, not only for the initial dose. Regarding cardiac function, subjects are assessed at the time of enrollment onto PLAT-2, which will be done just prior to enrollment onto PLAT-3, and for Cohort C, at the time of enrollment onto PLAT-3. Subjects must have normal cardiac function, as defined per the protocol. Normal cardiac function must be documented prior to the test dose of T-APC. If a patient subsequently has cardiac toxicity with a decline in the parameters as defined in the

inclusion criteria, a repeat echocardiogram documenting return to normal function will be required prior to any additional infusions.

The protocol guidelines for management of hypotension are to encourage more aggressive use of vasopressors, and less reliance on fluid resuscitation, which may be less effective in the setting of severe CRS with capillary leak. Transient hypotension will initially be managed by intravenous fluid administration; patients with persistent hypotension will require transfer to the ICU for definitive medical treatment. If significant hypotension occurs during infusion of the T-cell product, the infusion should be immediately suspended. The specific guidelines, including how significant hypotension is defined and the treatment algorithm for significant hypotension, are delineated in the protocol. The investigators noted that a search of the protocol did not locate the parameter of greater than 40 minutes after onset of hypotension prior to initiation of vasopressor support (as mentioned by the reviewer).

Assent will be required for subjects age 13 and older. The investigators will respect the child's dissent if it is clear that the child does not want to participate in the study. If parents wish to pursue therapy in spite of their child's dissent to participate, the study team will seek an alternative or work with the child through the pediatric advanced care team conference structure to understand their concerns and address them if possible. If the child's dissent is related to any therapy in general, and not the research specifically, the investigators could try to help decrease their anxiety around therapy and pursue treatment on- or off-study.

The investigators consider the lymphodepletion chemotherapy regimen and the frequency of bone marrow aspirations/biopsies and LPs to be standard of care in this patient population. Of the 42 treated subjects on PLAT-2, none have been ejected due to chemotherapy toxicities from lymphodepletion; this toxicity is therefore not expected to be an issue going forward. Subjects must be well enough to proceed with lymphodepletion chemotherapy. A statement will be added to the consent to clarify that there is a small risk that patients may be taken off study for chemotherapy-related toxicity prior to receiving CAR T cells.
The consent will also be revised to include additional background information on other trials to date, to explain that this is an experimental research study, and that the trial will be the first in humans to use T-APCs to "boost" the effect of the CAR T cells. The consent will also better describe the potential risks of the research; explain that while some subjects may benefit from the study intervention, for some patients, the CAR T cells will not work; and clarify what "withdrawal" from the protocol means, as suggested by the reviewers.

Dr. Jensen clarified that it is standard policy of Seattle Children's Hospital to provide and cover the medical costs of any care to a patient who is injured as a result of participating in a trial. The statements in the consents submitted for review are from older versions of the documents and will be removed and replaced with the correct information.

The word "vaccine" will be replaced with "T-APC cell product," and the word "therapy" will be replaced with "potential therapy" in the consent, as suggested.

The investigators will revise the ICDs to address the additional comments of the reviewers.

2. Responses to RAC Discussion Questions

The proposed trial is a single-center study at Seattle Children's Hospital. To assure equal access to this and other trials at this site, the pediatric hematology-oncology faculty are regularly briefed on upcoming and open trials through educational activities. In addition, there is a centralized patient referral database, and weekly meetings are held in which all incoming patient referrals are discussed to help manage enrollments. For patients to be eligible for PLAT-3, they must have been already enrolled in PLAT-2; thus, any patient on PLAT-2 that meets the eligibility criteria for PLAT-3 would be presented with the proposed trial as a possible option. In terms of enrolling subjects on a first-come/first-serve basis, if two patients meet the inclusion criteria for a protocol, but expressed interest in the study on separate days, and only one spot on the trial is open, the spot would go to the first patient to inquire about the study.

The protocol materials will be revised to clarify that the study involves two different modified T-cell products, T-APCs and CAR T cells. In addition, the protocol and Appendix M will be revised to be inclusive of the relevant portions of PLAT-2. Dr. Jensen noted that designing the proposed study has been challenging, given that the team has not previously had a trial that connects to another IND study.

Dr. Jensen acknowledged that there is not sufficient data at this point to know how to precisely parse out the relapsers from the non-relapsers. While factors such as disease burden, T-cell persistence, and BCA can be informative, there currently is no predictive biomarker that will absolutely define which patients will have short-term persistence and a higher risk of relapse. The investigators initially discussed giving T-APCs to everyone enrolled in the proposed trial because there are no a priori data to suggest that administration of T-APCs would harm a patient with CAR T cells. This plan was reconsidered with the realization that the approach would not work with the statistical design for PLAT-2. The study was therefore designed so that patients identified for Cohort A will be removed from PLAT-2 before lymphodepletion and CAR T cells; these subjects will be replaced in PLAT-2. Subjects in Cohort B or C will have lymphodepletion and CAR T cells under PLAT-2. Subjects moved from PLAT-2 to Cohort B will be considered a failure under PLAT-2 in terms of persistence. Subjects for whom a response evaluation is done before moving to PLAT-3 will be considered as evaluable for efficacy; otherwise, the subject is considered a failure. The investigators had originally planned to use MRD-negative remission by day 21 as the primary endpoint for the second phase of PLAT-2, but subsequently added the duration of B cell aplasia as a secondary endpoint for PLAT-02, which will count against PLAT-2 when patients are removed from PLAT-2 to Cohorts B and C in PLAT-3. Dr. Gardner noted that the second phase of PLAT-2 is intended to enroll about 70 patients, and Cohort A will enroll 10 patients. The investigators are aware of the potential for bias and will take these factors into account in the statistical analysis plan.

The investigators had expected to see CD19-negative relapses in patients with ongoing long-lasting persistence, but the timing and duration of this outcome have varied by patient. For example, one patient didn't relapse until after 8 months, but others relapsed at between 2 and 4 months. Anecdotal data suggest that some CD19-negative relapses occurred after relatively short periods of CAR engraftment and B cell aplasia, while delayed relapses have occurred after prolonged periods of B cell aplasia. In general, however, CD19-negative relapse is an early post-CAR T cell dosing event. It is not clear whether the subset of patients with long-term CAR T-cell persistence would benefit or be harmed from the planned intervention. The technology that will be used in PLAT-3 will not address CD19-negative relapses. The team expects to submit another protocol to the RAC in the next few months in which a dual-specific product that simultaneously targets CD19 and CD22 will be assessed to determine whether that intervention can help address the issue of CD19 relapse.

The clinical research program is set up so that Seattle Children's Hospital is the institutional sponsor of the study and Dr. Jensen is the institute/sponsor representative. The clinicians serve as the Principal Investigator, which is Dr. Annesley's role on the proposed trial, and the Lead and Associate Investigators. The protocol identifies the sponsor as Seattle Children's Research Institute and Dr. Jensen as the Project Lead. These roles and titles can be clarified to reflect the IND sponsor. Dr. Jensen pointed out that federal regulations allow sponsors to close protocols based on considerations that may be related to the FDA review or other factors. A sponsor should never intercede at the level of an individual patient. The protocol will be revised to clarify this distinction.

Dr. Jensen agreed with the reviewers as to the complexity of the study design and in introducing this technology for the first time in this patient population. The team felt that fixed dosing would be a reasonable way to proceed because the T-APCs are not bioactive when infused. In studies in which monkeys are given T-APCs only (without CAR T cells), there is no biological or clinical activity related to infusion of these cells. Thus, the bias is that the variable that might have the most impact on toxicity would be the magnitude of engraftment of CAR T cells at the time that the T-APCs are delivered, which is a variable that cannot be controlled.

The fixed dosing for children who weigh less than 25 kilograms, the smallest child that can be enrolled based on being able to undergo apheresis, is 25 million cells/kg. For larger patients, the dose will be 20

million cells/kg, which is well below the 100 million cells/kg dose that was found to be safe in the macaque studies. The decision therefore was to be conservative in choosing the flat doses for the proposed trial.

Because more extensive sampling and testing can be done in animals than in humans, there are extensive data sets from the NHP studies in particular. This includes very detailed and quantitative sampling of blood, CSF, lymph nodes, and bone marrow, with a focus on individual cytokines that may be driving the CRS and on cellular toxicities and CAR T cells in the CSF that are associated with neurotoxicity. To date, nothing from the detailed analysis of the macaque data stands out as far as toxicity, either pre- or post-T-APC infusion, to suggest that the timing of the infusions is dangerous in this cohort of non-human primates. These findings have helped guide the team in determining how to set up the proposed trial, including the decision to not give T-APCs before CRS but rather, to wait until after there is evidence of CRS when cells are still present. With an exploratory protocol such as this, it is best to chart out the most reasonable initial approach and then study each patient in real time as closely and as carefully as possible. In certain instances, it may be prudent or necessary to temporarily pause the protocol and adjust some of the parameters based on these real-time data instead of proceeding through an entire protocol in which adjustments should have been made earlier.

Regarding the importance of normal organ function at study entry, Dr. Jensen noted the team's experience to date with 42 children treated under PLAT-2. There have been significant medical management issues for some of these patients, but no toxic fatalities. The children who will participate in the proposed trial have gone through multiple years of multi-agent drug therapy. Most have had an allogeneic transplant and now find themselves with refractory disease. These patients present a challenge because none of them have normal physiology at this point based on their prior history of cytotoxic chemotherapy and radiation. While subjects are not being lost to toxic death, they represent a highly vulnerable and debilitated population. The investigators will consider the recommendations for functional re-assessment and restaging of patients before each T-APC dose as an added safeguard.

The investigators will consider a higher Karnofsky score for study eligibility. Many of the inclusion/ exclusion criteria for PLAT-2 and PLAT-3 are the same, including the cutoffs for the Lansky and Karnofsky scores, both of which are 50 percent. Dr. Gardner noted that several other criteria need to be met, including some related to organ function, and that it would be rare to see a patient with a Karnofsky score of 50. Those with the lowest performance scales are usually also the patients with the greatest chronic pain issues.

The proposed study has two consent forms, one for Cohorts A and C and one for Cohort B. The patients in Cohorts A and C receive CAR T cells and T-APCs under PLAT-3. Patients in Cohort B will have already received their CAR T cells on PLAT-2 and will receive only T-APCs on PLAT-3. Regarding the question to be more transparent in the consents regarding the research risks, Dr. Gardner clarified that the consent form for Cohort A and C includes three paragraphs describing the toxicity of CRS, including that CRS can be life-threatening and that the patient could be admitted to the ICU; in addition, there is a separate paragraph on neurotoxicity. Additional information on the tests, procedures, and interventions that could be done to manage CRS is also provided.

Dr. Gardner clarified that the intention of the supportive care section of the protocol is to say that each patient will receive the type of supportive care that is appropriate for them, per institutional guidelines.

The reference in the consent to reduced ability of the heart to pump is described in greater detail in narrative form (vs. what is listed in the listing of the risks and side effects table). It does not refer to cardiac arrest, and the consent explains that medications and transfer to the ICU may be required to treat this side effect.

The FDA requested that language regarding contraception, pregnancy, and nursing be incorporated into the consent for PLAT-2, and so the investigators also included it in the PLAT-3 consents.

The investigators will clarify the language in the protocol regarding how SAEs are defined and which SAEs (or categories/types of SAEs) should be reportable, as suggested by Dr. Zoloth.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Clinical and Trial Design

- Consider focusing on enrollment of research participants with a higher Karnofsky score than 50.
- Carefully consider the parameters for reporting PLAT-02 and PLAT-03 results, and the potential for selection biases.
- Because cardiac dysfunction can be exacerbated in the stress of cytokine release syndrome, it is suggested that the entry criteria for PLAT-03 for cardiac function parameters should be within the normal range.
- Explicitly clarify, in Appendix M, protocol body, and informed consent documents, that research participants will receive two separate gene transfer products.
- Suggest clarifying that the sponsor will pay for medical costs that are due to injury on this trial.

Ethical, Legal, and Social Issues

- Suggested changes to the consent form include:
    - Ensure that the different consent and assent forms are clear and appropriate, for different cohorts/populations of research participants (including minors and their guardians).
    - Consider removing graphs from the informed consent documents and instead using narrative paragraphs.
    - Assent forms for minors over 12 should clarify that subjects can freely refuse participation.
    - Clarify the risks of cytokine release syndrome, as relevant.
    - Consider modification of the sections regarding pregnancy and nursing babies, in light of the clinical situation of research participants.
    - Clarify that withdrawal from the study is not equivalent to withdrawal from the intervention, and clarify the duty of the investigators to follow research participants in an ongoing way.
    - Regarding terminology, change "doctor/patient" to "investigator/research participant" throughout.
    - Clarify, even in questions, that there is no anticipation of benefit to research participants.
    - Clarify that T-APCs themselves may persist, and the implications of that persistence are unknown.
    - Clarify the criteria for withdrawal from the study, and clarify that the sponsor will not independently add or remove individual research participants from the study.
    - Clarify that certain events (e.g., re-hospitalization, drug addiction) are considered serious adverse events.

G. Committee Motion 6

Dr. Donahue summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Donahue requested a vote, and the RAC approved these summarized recommendations by a vote of 10 in favor, 0 opposed, 0 abstentions, and 1 recusal (Dr. Whitley).

XIII. Closing Remarks and Adjournment

Dr. Tucker provided an update on the status of changes to the RAC review process that were proposed in October 2015, followed by the open comment period that closed in December 2015. The comments have been reviewed, and the changes, which are based largely on the original proposal previously reviewed by the RAC, have been finalized and went into effect April 27, 2016. Per the Institute of Medicine's (IOM's) proposal, an oversight body such as an IRB will make the recommendation for RAC review. Those recommendations will be based on criteria outlined in the IOM report, that is, that the proposed intervention is sufficiently novel or has a safety characteristic that should be evaluated by this committee. In some cases, the NIH Director could designate a proposal for RAC review if the therapy poses a significant safety, ethical, or scientific consideration. Any protocols that may be reviewed at the next RAC meeting will be operating under the updated policy. Details of the new process will be included in the upcoming annual orientation prior to the next meeting.

The NIH will continue to monitor the gene therapy landscape and will still receive all submissions from all investigators, including, for example, information on SAEs and novel applications of previously used vectors. As currently done, that information will be incorporated into the existing protocol registry database and will remain accessible to the RAC (via GeMCRIS). A summary of why a specific protocol is not recommended for in-depth RAC review will continue to be provided. The number of RAC meetings will not necessarily change once the new process is in place. RAC members will continue to have standing meeting time to review protocols as needed and to address other issues such as review of FDA proposals and consideration of emerging issues in the field.

Dr. Tucker thanked all present for making her first meeting as Executive Secretary of the RAC productive, lively, and interesting. She thanked the outgoing RAC members again for their service and noted that she is looking forward to working with the Committee in the future.

Dr. Whitley thanked the RAC members and the BBEBP staff and adjourned the June 2016 RAC meeting at 12:40 p.m. on June 22, 2016.

[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, they are not considered final until approved by the NIH Director.]

_____
Jessica Tucker, Ph.D.
RAC Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and the following Attachments are accurate and complete.

This Minutes document will be considered formally by the RAC at a subsequent meeting; any corrections or notations will be incorporated into the Minutes after that meeting.

Date: _____

_____
Richard Whitley, M.D.
Acting Chair, Recombinant DNA Advisory Committee

Attachment I:
Recombinant DNA Advisory Committee Roster

Chair

KIEM, Hans-Peter, M.D., Ph.D.
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José Carreras/E. Donnall Thomas Endowed Chair for Cancer Research
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Executive Secretary

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Attachment II:
Public Attendees

(This list includes only individuals who are not identified elsewhere in this document. It does not include three individuals whose names are illegible on the sign-in sheets.)

Alexander Astrakhen, bluebird bio
Sandra Bridges, National Institute of Allergy and Infectious Diseases, NIH
Ken Cannon, 3D Communication
Tim Chan, Intrexon
Tandy Chandler, National Human Genome Research Institute, NIH
Reed Clark, Dimension Therapeutics
Eric Crombez, Dimension Therapeutics
Iulia Diacenu, bluebird bio
Rebecca Hang, National Human Genome Research Institute, NIH
Eben Kirsky, Princeton University
Amy Lankford, Intrexon
Cat Leonard, 3D Communication
Julie Lin, Dimension Therapeutics
Kay Miller, Ohio University
Molly Roberts, Dimension Therapeutics
Rutul Shah, Intrexon
Arvind Suresh, Genetics Experts News Service
Michael Swartz, Intrexon
Don Tropez, Ohio University
Barbara Vance, University of Pennsylvania
Ramjay Vatsan, FDA
Schuyler Vinzant, Intrexon
Sam Wadsworth, Dimension Therapeutics
Todd Wiegile, 3D Communication
Shannon Williams, Intrexon

Attachment III:
Abbreviations and Acronyms

AAV	adeno-associated virus
AAV8	adeno-associated virus serotype 8
ADA-SCID	adenosine deaminase deficiency-severe combined immunodeficiency
AE	adverse event
ALL	acute lymphoblastic leukemia
ALT	alanine transaminase
AML	acute myeloid leukemia
B-ALL	B-cell acute lymphoblastic leukemia
BBEBP	Division of Biosafety, Biosecurity and Emerging Biotechnology Policy
BCA	B cell aplasia
bp	base pair
CAR	chimeric antigen receptor
Cas9	CRISPR-associated protein 9
CCR5	chemokine receptor type 5
CD19t	CD19-encoding transgene
CED	convection-enhanced delivery
CHOP	Children's Hospital of Philadelphia
CISC	UPenn Conflict of Interest Standing Committee
CLL	chronic lymphocytic leukemia
CNS	central nervous system
COI	conflict of interest
CPHS	Committee for the Protection of Human Subjects
CRISPR	clustered regularly interspaced short palindromic repeats
CR	complete remission
CRM	continual reassessment model
CRS	cytokine release syndrome
CVPF	UPenn Clinical Cell and Vaccine Production Facility
CY/FLU	cyclophosphamide/fludarabine
ddPCR	digital droplet PCR
DLT	dose-limiting toxicity
DMC	Data Monitoring Committee
DSMB	Data and Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
EGFRt	truncated epidermal growth factor receptor
EOHW	Employee Occupational Health and Wellness
FACS	fluorescence-activated cell sorting
FCOI	financial conflict of interest
FDA	U.S. Food and Drug Administration
GFR	glomerular filtration rate
GBM	glioblastoma multiforme
gc	genome copies
GeMCRIS	Genetic Modification Clinical Research Information System
GLP	good laboratory practice
GMP	good manufacturing practice
gRNA	guide RNA
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HCC	hepatic cellular carcinoma
HER1t	truncated human epidermal growth factor receptor
HLA	human leukocyte antigen
IBC	Institutional Biosafety Committee
ICD	informed consent document
ICOI	institutional conflict of interest
IgG	immunoglobulin G

IgH	immunoglobulin H
IL-2	interleukin-2
IL-6	interleukin-6
IND	Investigational New Drug
indel	insertion or deletion mutation
IOM	Institute of Medicine
IPHP	Institutional Product Handling Plan
IRB	Institutional Review Board
IRES	internal ribosome entry site
IV	intravenous
KO	knockout
Lm	*Listeria monocytogenes*
LD	lymphodepletion
LP	lumbar puncture
LPD	lymphoproliferative disorder
LTFU	long-term follow-up
mAb	monoclonal antibody
MDACC	MD Anderson Cancer Center
MED	minimal effective dose
MRD	minimal residual disease
mRNA	messenger RNA
MTD	maximum tolerated dose
NHP	nonhuman primate
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NOAEL	no-observed adverse effect level
NSG	NOD/scid/γcnull mice
NYCE	NY-ESO-1 CRISPR Edited
OBD	optimal biological dose
OD	NIH Office of the Director
OHSU	Oregon Health and Science University
OSP	NIH Office of Science Policy
OTC	ornithine transcarbamylase
PDCD1 or PD-1	program cell death protein-1
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PLAT	pediatric leukemia adoptive therapy
PV	poliovirus
PI	principal investigator
rAAV	recombinant adeno-associated virus
RAC	Recombinant DNA Advisory Committee
RCL	replication-competent lentivirus
SAE	serious adverse event
SIN	self-inactivating
SOP	standard operating procedure
spf^{ash}	sparse fur
SPRT	Sequential Probability Ratio Test
scFv	single-chain variable fragment
T-APC	T-antigen presenting cell
$TCID_{50}$	50 percent of the tissue culture infectious dose
TCR	T cell receptor
TCR^{endo}	endogenous TCR
TLS	tumor lysis syndrome
TRAC	T cell receptor-α
TRBC	T cell receptor-β
Treg	regulatory T cell
UCD	urea cycle disorders
UCSF	University of California San Francisco

ULN	upper limit of normal
UPenn	University of Pennsylvania
UTIMCO	University of Texas Investment Management Company
vg	vector genomes
xGVHD	xenogeneic GVHD
ZFN	zinc finger nuclease

Appendix A:
Public Comments

[No public testimony was provided at the June 2016 RAC meeting.]